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MORPHOGENESIS DURING REGENERATION IN AN ENTEROPNEUST*

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THE capacity of the enteropneusts for regenerating lost parts of the body was first noticed by Spengel (1893) in *Glossobalanus minutus*. Willey (1899) made notes on the regeneration in *Ptychodera flava*, while Asheton (1908) observed fragments of *Dolichoglossus pusillus* regrowing into wholes. Since Dawydoff (1907, 1909) gave an account of the phenomenon in *Glossobalanus minutus*, no noteworthy contribution to our knowledge has been made. Hence it was of interest to study the morphological and histological changes during regeneration in *Ptychodera flava*, especially as recent work on regeneration has emphasized the significance behind the resemblance to processes of development.

Material and Methods

Large numbers of *P. flava* were collected from the intertidal region at Krusadai Island in the Gulf of Manaar. Each animal was cut into four or five pieces and reared in laboratory containers in fresh sea water, some with sand and others without it. The water was changed twice a day. Some of the regenerating fragments of the enteropneust were fixed every 24 hours in alcoholic Bouin's fluid or in corrosive acetic and sectioned at 6 to 8/μ in thickness. Heidenhain's iron hæmatoxylin with eosin was generally used for staining.

Results

External changes during regeneration.—The animals were cut into four pieces : 1. The proboscis and the collar; 2. The branchial region including the œsophageal region; 3. The hepatic region including a part of the abdominal region, and 4. The rectal region along with the later half of the abdominal region.

In the first case no regeneration was observed and the bit continued to live for a considerable period. In the second case the anterior end as well as the posterior remained open, and at the anterior end a proboscis developed mid-dorsally in about ten days. In the case of the third and fourth pieces the anterior end closed and a tiny proboscis was distinguished at the mid-dorsal part of the closed front end on the fifth day. The collar was formed much later and was noticed on the 11th day. In the first three cases, the posterior end healed over by a growing together of the epidermis and the gut-wall. Of the rectal bits 1 cm. long, about 20% perished, while the remaining 80% were remarkble in regenerating a proboscis as swiftly as the much longer hepatic and abdominal portions of the animal.

* Contribution from the University Zoology Laboratory, Madras.

Organogenesis.—The closure of the anterior end was brought about by a growing together of the edges of the ectoderm and those of the gut-wall (Fig. 1).

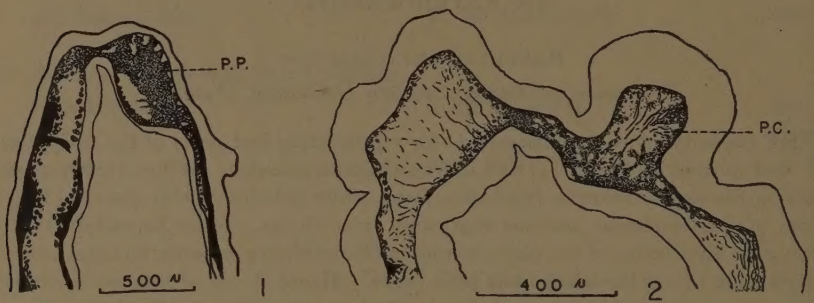

Fig. 1. Sagittal section of anterior end of a regenerating piece. 72 hours after amputation. Anterior end to the top and dorsal to the right. P.P., proboscis primodrium.

Fig. 2. Sagittal section of anterior end of a regenerating piece. 120 hours after amputation. Orientation same as before. P.C., proboscis cœlom.

This inward growth, is by the multiplication of the ectoderm cells and the rapid division of the endoderm cells of the gut-wall in the wounded region. When the breach had been completely closed, the appearance of an increasing number of cœlenchyme cells was noticed. That these elements, normally found in the cœlomic fluid of the trunk region, migrated to the regenerating region could be inferred from serial sections. These cœlenchyme cells together with the ectoderm and endoderm cells healing the end, form the rudiment from which different organs of the proboscis are formed.

The proboscis.—The *cœlom* of the newly forming proboscis is derived from the existing trunk cœlom by an evagination at the anterior mid-dorsal part, and by a migration of cœlenchyme elements from the trunk cœlom into this newly formed rudiment (Fig. 2). The muscle and connective tissue elements of this diverticulum undergo a process of degeneration and are absorbed by the free cœlenchyme cells, which thus grow in size and appear undifferentiated, round and granular. These cells, which were phagocytic, now become "embryonic" in character. Some occupy positions along the periphery and arrange themselves into a layer enclosing the proboscis cavity, while some others still remain in the interior of the cavity (Fig. 2). After this, a redifferentiation of these cœlenchyme cells commences. Some of the round embryonic cells in the central and peripheral regions become elongated and constitute the new muscle fibres, from which the longitudinal muscle system of the proboscis is formed.

The *basal membrane* is formed as a continuation of the old membrane and is developed as two lamellæ, one derived from the epidermis and the other from the cœlomic layer close by. No nuclei were seen in the basal membrane in *Ptychoderc flava* and the membrane always presented a uniform structureless appearance

The *proboscis skeleton* is developed as a local thickening of the basal membrane, between the stomochord and the ventral epidermis (Fig. 7). As in ontogeny, it is formed as a stratified structure from the epithelia of the epidermis and the stomochord. There is no connection between the rudiment of the skeleton and the cœlenchyme cells.

The *cardiac vesicle* is cut off from the posterior part of the proboscis cœlom by an inpushing of the dorsal wall of the cœlom (Fig. 8). Thus a closed sac lined by a layer of cœlenchyme cells, and containing a few cells in its cavity is formed. These cells later differentiate into the muscle and connective tissue fibres.

The *stomochord* is known to arise as a mid-dorsal evagination of the anterior part of the gut, now closed (Dawydoff, 1909). Besides such a method of forma-

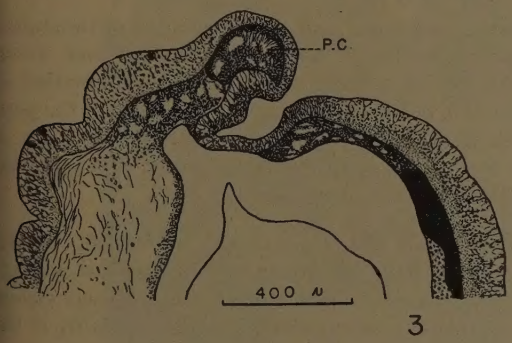

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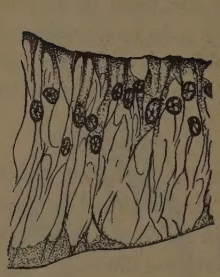
Fig. 3. Sagittal section of a regenerating piece. 144 hours after amputation. Early stage in the formation of the stomochord through metaplasia. Orientation as before, but with the dorsal side to the left.

Fig. 4. Epidermal epithelium before metaplasia.

tion, a remarkable instance of metaplasia was observed in the present form. Before the epithelium of the anterior blind end of the gut is differentiated into the vacuolar tissue, an ectodermal invagination is formed at the anterior blind end of the regenerating bit, ventral to the proboscis rudiment. The epidermal epithelium of the mid-dorsal part of this invagination (Fig. 4) now rapidly differentiates into a vacuolar tissue (Fig. 5) similar in all respects to the buccal epithelium. This differentiated epidermal epithelium now gives rise to a mid-dorsal diverticulum which pushes into the proboscis cœlom and finally forms the stomochord (Figs.3,6). Thus a structure which as a rule is derived from the endoderm, is in the present case formed from the ectoderm through a process of metaplasia. In either case the stomochord shows a clear cavity, which is sometimes extraordinarily large. The mouth breaks open only after the differentiation of this epidermal epithelium is completed.



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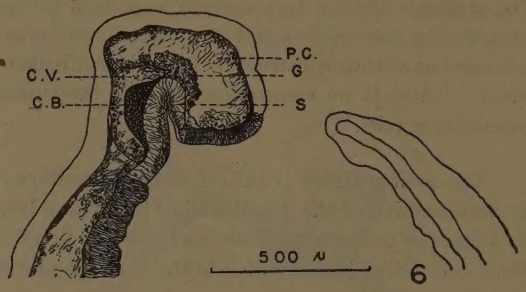

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Fig. 5. Epidermal epithelium after metaplasia.

Fig. 6. Sagittal section of anterior end of a regenerating piece. 168 hours after amputation. Orientation same as for Fig. 3. C.B., central blood space; C.V., Cardiac vesicle; G., glomerulus; P.C., proboscis cœlom; S., stomochord.

The *glomerulus* is differentiated immediately after the formation of the stomochord (Figs. 3, 6). The peritoneum covering the anterior, lateral and dorsal walls of the stomochord becomes folded. Simultaneous with the formation of these folds, histological differentiation of the cœlomic cells of this part of the peritoneum takes place. Many cells lining these folds become cuboidal and are of a glandular character. Besides these, cells of an excretory nature, with yellowish granular content in their cytoplasm could be distinguished.

By about this time the *proboscis canal* and *proboscis* pore are formed. In the posterior dorso-lateral part of the proboscis an ectodermal invagination appears and grows in the direction of the posterior blind end of the proboscis cœlom (Fig. 9). The peritoneum of the proboscis cœlom which is in the proximity of the ectodermal invagination shows no differentiation. The ectodermal invagination finally opens into the posterior dorso-lateral part of the proboscis cœlom. The cells of this ectodermal tube are ciliated. Thus the peripheral part of the proboscis canal as well as the proboscis pore are entirely formed from the ectoderm as in ontogeny.

Immediately after the closing of the anterior end, the nerve fibre layer is continued over the closed end beneath the ectodermal epithelium. This nerve fibre layer is thickened at the posterior end of the proboscis.

The *collar cœlom* of the regenerating piece is formed behind the proboscis rudiment as a pair of independent constrictions from the right and left cœlomic cavities (trunk cœlom). The disintegration of the connective tissue and muscle cells of these constrictions and their substitution by redifferentiation of the cœlenchyme elements are similar to those observed in the proboscis rudiment.

The *collar canals* also are formed by invagination of the ectoderm on either side. These invaginations are well differentiated even before the first gill-slit is formed. The collar canals open to the exterior along with the first pair of gill-slits (Fig. 10).

PLATE I

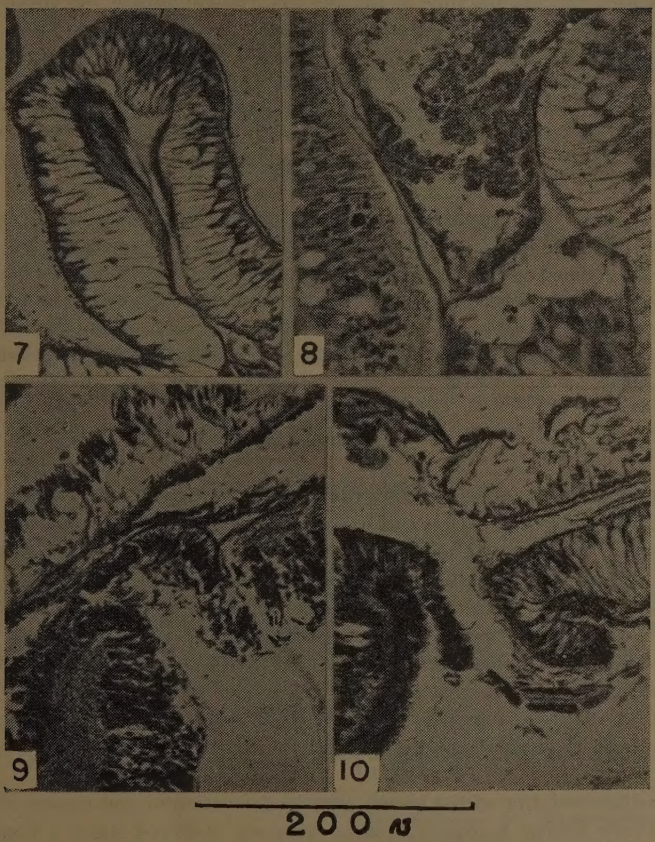

Fig. 7. Longitudinal section through the stomochord, showing the formation of the skeleton during regeneration.

Fig. 8. Sagittal section through the proboscis rudiment, showing the formation of the cardiac vesicle.

Fig. 9. Longitudinal section through the proboscis rudiment showing the formation of the proboscis pore.

Fig. 10. Section showing the collar pore.

The anterior part of the *alimentary canal* is closed sometime after the amputation (Fig. 1). The whole alimentary canal is lined by a thin epithelium of cylindrical cells with richly granular protoplasm. In later stages, a remarkable and rapid histological differentiation takes place in the anterior part of this closed gut, resulting in typically vacuolated tissue, with elongated cells containing little protoplasm. This epithelium resembles the buccal epithelium of the normal enteropneust. From this redifferentiated anterior part are derived the stomochord and the buccal

epithelium. The mouth opening is formed by an approximation of the gut and the epithelium of the epidermis at the anterior ventral part of the blind end of the gut.

In the mid-dorsal region of the collar, the nerve fibre layer is thickened to form the nerve cord of the collar by a process of sinking in. As in ontogeny, the margins of the mid-dorsal groove close over giving rise to the tubular collar cord which is in connection with the ectoderm along its whole length for a considerable period.

The trunk.—The formation of the *branchial region* starts late in regeneration, after almost all the other organs are differentiated. Just as in ontogeny, the gill pouches are formed as outpushings of the gut immediately behind the buccal region, which on coming in contact with the ectoderm open to the exterior by means of the crescent-shaped gill-slits with the concavity of the crescent on the dorsal side. The epithelium lining these gill rudiments consists of short cylindrical cells bearing cilia.

Discussion

In *Glossobalanus minutus*, Dawydoff (1909) noticed nuclei within the basal membrane during regeneration and hence considered it as a specialized part of the mesenchyme. However, I could not find any nucleus in the basal membrane of *Ptychodera flava* either in ontogeny (Rao, 1954) or during regeneration. Dawydoff (1909) also observed an immigration of cœlenchyme cells into the fold of the basal membrane during the formation of the proboscis skeleton and considered that these cells take part in its formation. The present investigation reveals that the cœlenchyme elements take no part in the formation of the skeleton. It is likely, as Horst (1927-1939) has observed, that Dawydoff referred only to the formation of the chondroid tissue and not the skeleton.

The differentiation of the tissues and the formation of new organs in regeneration are very similar to corresponding processes observed when a young animal develops from the fertilized egg. Thus the differentiation of the proboscis takes place first, then the collar and lastly the trunk. In both cases the body surfaces, ventral or dorsal, once formed appear fixed. The formation of the basal membrane, the proboscis and collar canals and their pores, the proboscis skeleton, the glomerulus, the gill-slits as well as the nerve cord of the collar, are all similar in both ontogeny and regeneration. But there are certain differences. The cœlom of the regenerated parts is derived from the already existing trunk cœlom of the regenerating piece and not from the gut. The cardiac vesicle of the regenerated proboscis is formed from the cœlom and not from the ectoderm or mesenchyme as during ontogeny. But the most striking difference is observed in the derivation of the stomochord. Out of the 16 cases studied, in three it was formed from the ectoderm cells freshly formed to cover the wound, whereas in the majority of cases it was formed from the endodermal cells which had multiplied to cover the cut end. It is probable that the ectodermal cells of the healed region as well as the endodermal cells

were of a dedifferentiated embryonic character. The formation of the stomochord from the ectoderm, through metaplasia, supports the view that it is not the tissue which is ' *actu* ' regenerating but that regeneration is a consequence of the potentiality immanent in the organism. The great similarity between ontogeny and regeneration justifies the belief that regeneration is to be regarded as a primary function of living matter, just as is normal development.

Summary

1. The process of regeneration in the adult *Ptychodera flava* has been studied by transecting animals and rearing the pieces in the laboratory.

2. Histogenesis and organogenesis during regeneration are very similar to these processes in ontogeny, although a remarkable exception in the formation of the stomochord from the epidermis due to metaplasia is observed.

3. The great similarity between ontogeny and regeneration suggests that regeneration is a primary function of living matter, just as is normal development.

Acknowledgment

I am greatly indebted to Dr. C. P. Gnanamuthu, Director, University Zoology Laboratory, Madras, for his encouraging criticism during the course of this investigation. My thanks are due to the Assistant Director of Fisheries (Marine Biology), Calicut, for all the laboratory and collection facilities given me at the Marine Biological Station on the Krusadai Island in the Gulf of Manaar.

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THE CENTRAL NERVOUS SYSTEM OF *PANULIRUS POLYPHAGUS* (HERBST)

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THE central nervous system of *Panulirus polyphagus* consists as in arthropods in general of a ganglionated chain which extends from the end of the abdomen to the head. In the thorax and abdomen the chain remains ventral to the alimentary canal, while in the head the two component cords of the chain separate from one another and after circumventing the œsophagus terminate on its dorsal side in the supra-œsophageal ganglion or brain. Along the ventral chain there are six abdominal and two thoracic ganglionic masses. Associated with the central nervous system is a visceral part which innervates the visceral organs (Fig. 1 and 2).

The supra-œsophageal ganglion or brain.—Embedded in a thick cushion of fatty tissue and lying immediately below the middle point of the posterior margin of the narrow forward extension of the carapace is a white mass of nervous tissue, the brain. Antero-dorsally viewed, it appears as a trapezoid body with an anterior smaller and a posterior larger face. The anterior face is distinctly divided into three pairs of areas which perhaps indicate three pairs of ganglia. In Palæmon it has been described that the three parts of the brain, the proto-cerebrum, the deuto-cerebrum and the trito-cerebrum are indistinguishably fused together and that its composite character can only be inferred from the fact that three pairs of nerves arise from it to innervate respectively the eyes, the antennules and the antennæ. In Panulirus these three divisions of the brain appear to be distinguishable owing to the three pairs of ganglia visible from its antero-dorsal aspect. Of these three pairs of ganglia, one pair is situated in the upper half of the brain and this pair probably represents the proto-cerebrum (proto-cerebrum and archi-cerebrum fused). The remaining two pairs are situated in the lower half of the brain, one of each pair on either side of the middle line ; of these the inner probably represents the deuto-cerebrum (meso-cerebrum) and the outer, the trito-cerebrum (meta-cerebrum). There is no such differentiation on the postero-ventral surface of the brain.

Three major pairs of nerves originate from the brain *viz.*, the antennary, antennulary, and the optic nerves. Besides these, there are several smaller pairs originating from the brain.

The optic nerves.—These are a pair of stout nerves which arise from the dorsal side of the brain one on either side. Each nerve passes slightly upwards and outwards and enters the eyestalk of its side to innervate the retinulæ.

The ophthalmic nerves.—They are a pair of slender motor nerves which arise close to the origin of the optic nerves one on either side of the brain. Each runs upwards and outwards for a short distance and enters the eystalk of its side to innervate the occular muscles,

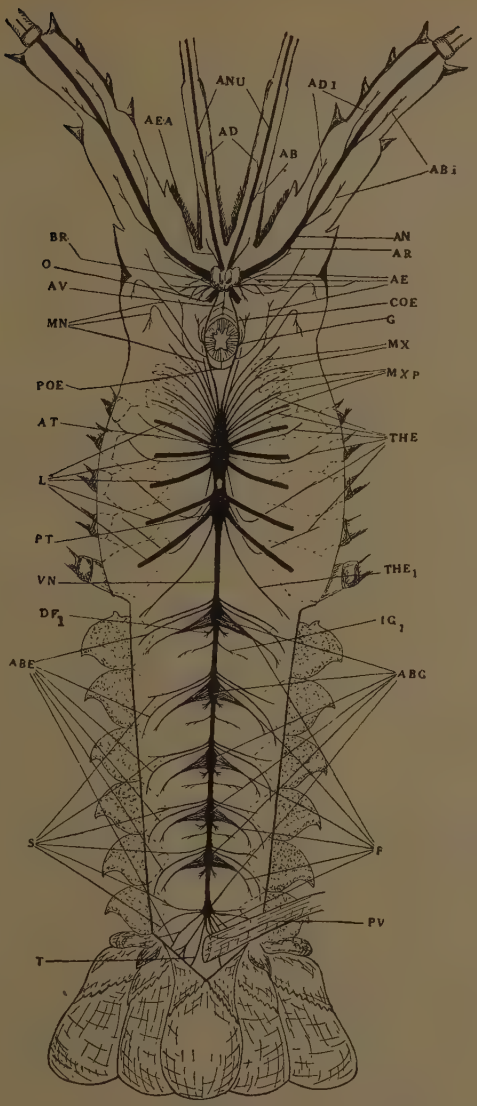

Fig. 1. The Central Nervous System of Panulirus.

AB, antennulary abductor nerve; AB1, antennary abductor nerve; ABE, abdominal extensor nerve; ABG, abdominal ganglia; AD, antennulary adductor nerve; AD1, antennary adductor nerve; AEA, antennulary elevator-abductor nerve; AE, antennary elevator nerve; AN, antennary nerve; ANU, antennulary nerve; AR, antennary rotator nerve; AT, anterior thoracic ganglion; AV, anterior visceral nerve; BR, brain; COE, circum-œsophageal commissure; DF1, first dorsal flexor nerve; F, nerves to the flexor muscles; G, ganglion on the circum-œsophageal commissure; IG1, first cordal nerve; L, nerves to the ambulatory appendages; MN, mandibular nerves; MXP, nerves to the maxillipeds; MX, maxillary nerves; O, optic nerve; POE, posterior œsophageal connective; PT, posterior thoracic ganglion; PV, posterior visceral nerve; S, nerves to the swimmerets; T, nerve to the telson; THE, thoracic extensor nerves; THE 1, thoracic extensor nerve of the first abdominal segment; VN, ventral nerve cord.

The antennulary nerves.—They are a pair of very stout cords originating from the ventral aspect and emerging out on view in front of the brain. Each nerve proceeds forwards and downwards for a short distance and then forwards and outwards again to the antennule of its side. It extends as far as the tip of the antennule. Along its course in the antennule the nerve gives out two pairs of lateral nerves, one pair in the proximal and the other in the distal segment. These nerves run forward and innervate the abductor and adductor muscles of the appendage which are disposed on either side. As the antennulary nerve gives out fibres to the abductor and adductor muscles of the antennule, as well as to the statocyst located in the basal segment and to the sensory bristles of the appendage, it is a mixed nerve with both motor and sensory fibres.

The antennulary elevator-abductor nerves.—They are a pair of slender nerves originating just above the place of origin of the antennulary nerves. Each of these nerves proceeds forwards and outwards along with the major antennulary nerve for a short distance. Then it divides into two branches; the inner branch farther proceeds upwards to innervate the elevator muscles, while the outer branch innervates the outer abductor muscles of the antennule.

The antennary nerves.—These are a pair of very stout nerve cords arising from the ventral side of the brain, and emerging in front of it one on either side between the optic nerve and the antennulary nerve of each side. Each of these runs obliquely outwards and forwards and after entering the coxocerite extends to the tip of the antenna. Along its course in the antenna, the antennary nerve gives out two pairs of lateral nerves. The first pair arises in the basicerite of the antenna and runs forward and innervates the abductor and adductor muscles which are disposed on either side in the proximal segment of the endocerite. The second pair of lateral nerves also arises from the antennary nerve in the proximal segment of the endocerite, but innervates the abductor and adductor muscles of the distal segment of the endocerite. As the antennary nerve gives out nerves to the abductor and adductor muscles of the antenna as well as to the sensory bristles of the antenna, it is a mixed nerve.

The antennary rotator nerves.—These are a pair of thin nerves which originate along with the antennary nerves on each side. Each nerve runs parallel to the antennary nerve on its outer side and after entering the coxocerite takes a forward and upward course over the antennary nerve to innervate the rotator muscle in the basicerite of the antenna.

The antennary elevator nerves.—These are five pairs of nerves of which four originate from the posterior side of the brain in-between the optic and the antennary nerves on each side, while the first pair originates along with the antennary nerves.

The first elevator nerve is a small slender nerve which after originating with the antennary nerve runs on its ventral side. It then takes a downward and forward course to innervate the inner ventral elevator muscle in the coxocerite.

The second elevator nerve runs parallel to the antennary rotator nerve and innervates the tendon of the two dorsal elevator muscles described below.

The third elevator nerve runs backwards and upwards and immediately divides into branches in the inner dorsal elevator muscle of the antenna.

The fourth elevator nerve takes a slightly more outward course than the third elevator nerve; then it branches to innervate the second dorsal elevator muscle. These two muscles are concerned with the elevation of the basicerite which is made to rub against the coxocerite, and as a result a drawn-out hoarse noise is produced.

The fifth elevator nerve is a slender nerve which originates from the posterior aspect of the brain between the nerves which supply the dorsal elevator muscles and the circum-œsophageal commissures. This nerve proceeds downwards and outwards as far as the recess in which the antennary gland is lodged. Approximately half-way along its course a branch arises from this nerve which takes a forward and outward course to innervate the outer ventral elevator muscle in the coxocerite. The main nerve terminates in the median ventral elevator muscle.

The circum-œsophageal commissures.—They are stout cords originating very close to each other from the posterior lower part of the brain. Each cord proceeds slightly outwards and then downwards along the side of the œsophagus and meets its fellow of the opposite side in the sub-œsophageal (anterior-thoracic) ganglionic mass situated in the thorax. Each commissure presents half-way along its course a small ganglion from which arise several nerves to innervate the various parts of the alimentary canal and liver.

A short distance behind the ganglion, a slender nerve arises from each circum-œsophageal commissure. This nerve proceeds forwards and outwards and ultimately innervates the adductor muscles of the mandible of the respective side.

The posterior œsophageal connective.—Close behind the œsophagus and immediately before the two commissures pass beneath the cephalic apodeme to meet in the sub-œsophageal ganglion, passes a short cord which connects the two circum-œsophageal commissures with each other. This has been often referred to as the posterior œsophageal connective by certain previous authors.

The thoracic ganglionic masses.—There are two ganglionic masses in the thorax. Each of them is embedded in a very thick cushion of fatty tissue. They lie below the arches formed by the mesophragma of the endosternites and immediately above the fused sternal plates—the plastron. The two ganglionic masses are so large that together they extend to one-third of the length of the thorax. The anterior mass is very much bigger than the posterior one. The former is elongated and roughly ovoid in shape while the latter is more or less round. The anterior limit of the anterior thoracic ganglionic mass lies in the same line as the bases of the third pair of maxillipedes, while its posterior margin lies in line with the bases of

the second pair of walking legs. The small posterior ganglionic mass is connected to the anterior mass by a pair of short cords, so that it lies close behind. It is at this place, that is between the two cords that the sternal artery descends to enter the sternal sinus. By comparing the nervous system of various types of crustaceans it is reasonable to conclude that each of these ganglionic masses is derived from the fusion of a number of ganglia. From the number of segments it innervates, it can be surmised that nine pairs of ganglia have fused to form the anterior mass and two pairs to form the posterior one.

The nerves arising from the anterior thoracic ganglionic mass are as follows :—

1. *The mandibular nerves.*—These originate on either side close to the junction of the circum-œsophageal commissures with the anterior thoracic ganglion. Each of these nerves runs forward for a short distance alongside the circum-œsophageal commissure of its side. It then separates from the latter and runs forward into the hollow of the apophysis of the mandible of its side. Here it branches into two and each branch divides into a large number of nerves. Of the two branches the posterior one innervates the adductor muscles of the mandible, while the anterior one ramifies along with the fifth elevator nerve in the median ventral muscle of the antenna. Therefore it seems that there must be some sort of co-ordination in the movements of the mandibles and the antennæ.

2. *The first maxillary nerves.*—These are a pair of very delicate short nerves which originate close behind the mandibular nerves. Each runs alongside the mandibular nerve of its side for a short distance and after separating from it runs forwards and slightly outwards to innervate the muscles of the maxilla.

3. *The second maxillary nerves.*—These are a pair of nerves originating close behind the first maxillary nerves. Each of these nerves proceeds forwards and outwards and branches profusely in the muscles of the second maxilla. These nerves are thicker than the first maxillary nerves and their great development is in correlation with that of the appendages which they innervate.

4. *The nerves to the maxillipedes,* 1-3.—These nerves originate in succession and each pair proceeds forwards and outwards for a short distance and ultimately innervates the muscles of the respective appendages.

5. *The nerves to the first pair of walking legs.*—These arise behind the nerves which innervate the third pair of maxillipedes. Each of the nerves proceeds outwards and forwards and passes between the endosternites 8 and 9 and finally enters the basal podomere of the walking leg of its side. It then divides into two branches which after innervating the muscles of that segment proceed farther and innervate the muscles of the other podomeres.

6. *The nerves to the second pair of walking legs.*—These are a pair of stout nerves originating behind the nerves which go to the first pair of walking legs. Each of

these nerves proceeds outwards, and after passing beneath the lateral arch between the mesophragma of the 9th and 10th endosternites and between the endosternites 9 and 10, enters the basal podomere of the second walking leg of its side. There it branches like its predecessor and innervates the second walking leg.

7. *The nerves to the third pair of walking legs.*—These are also a pair of stout nerves. They originate just behind the nerves which innervate the second pair of walking legs. Unlike the first two pairs, these nerves have to take a backward and outward course. Each of these after passing beneath the lateral arch formed by the mesophragma of 10th and 11th endosternites and between the endosternites 10 and 11 enters the basal podomere of the third walking leg and innervates that leg.

The nerves originating from the posterior thoracic ganglionic mass.—These are two pairs of stout nerves innervating the 4th and 5th pairs of walking legs.

1. *The nerves to the fourth pair of walking legs.*—These arise from the anterior region of the posterior thoracic ganglionic mass. Each of these nerves proceeds backwards and outwards, passes beneath the lateral arch formed by the mesophragma of the 11th and 12th endosternites and between the endosternites 11 and 12 and finally distributes itself to the 4th walking leg of its side.

2. *The nerves to the 5th pair of walking legs.*—These originate from the hind part of the posterior thoracic ganglionic mass. Each of this pair of nerves pursues a backward course for a short distance and passes outwards beneath the mesophragma of the 12th endosternite to the basal podomere of the fifth walking leg of its side in which it divides into many branches.

The Minor nerves originating from the thoracic ganglia.—Besides the above-mentioned major nerves several pairs of long and slender ones arise from the thoracic ganglionic masses. These nerves do not arise ventro-laterally as those mentioned before but somewhat from the dorsal side of the ganglionic masses. In all seven pairs of these nerves are traceable, though theoretically, eight pairs must be present. All these nerves except the last pair after passing before the endosternites pursue an upward course, and then piercing the pericardial membrane, innervate the extensor muscles in the pericardial chamber.

The first pair of these nerves originates between the 2nd and 3rd maxillipedal nerves. Each of these nerves passes forwards and outwards along with the second maxillipedal nerve of its side and then pursues an upward course, between the 6th and 7th endosternites.

The second pair originates between 3rd maxillipedal nerves and the 1st pair of walking legs. Each of them passes forwards and outwards along with the 3rd maxillipedal nerve and then pursues an upward course, between the 7th and 8th endosternites.

The third pair originates between the roots of the nerves to the 1st and 2nd pair of walking legs. Each of them passes outwards and forwards along with the nerve to the 1st walking leg, and then proceeds upwards between the 8th and 9th endosternites.

The fourth pair originates between the nerves to the 2nd and 3rd pair of walking legs, and proceeds outwards along with the nerves to the 2nd pair of walking legs. Each of these nerves pursues an upward course between the 9th and 10th endosternites.

The fifth pair originating behind the nerves to the 3rd pair of wallking legs, is the last pair arising from the posterior part of the anterior thoracic ganglionic mass. Each of these nerves takes a backward course on the side of the ventral nerve cords and proceeds as far as the posterior thoracic ganglionic mass between the nerves of the 4th and 5th walking legs. Then it takes an acute turn, runs forwards and outwards between the 9th and 10th endosternites to take an upward course.

The sixth pair originates between the nerves to the 4th and 5th walking legs, from the mid-dorsal part of the posterior thoracic ganglionic mass. These nerves pass backwards and beyond the nerves to the 5th pair of walking legs, and after taking a turn proceed forwards and outwards to pass between the 10th and 11th endosternites to pursue an upward course.

The seventh and the last pair originates close behind the roots of the nerves which innervate the 5th pair of walking legs. Each of these pursues a backward course and on reaching the first abdominal segment turns outwards and innervates the extensor muscles of the first abdominal segment.

The post-ganglionic thoracic nerve chain.—After giving rise to the posterior thoracic ganglionic mass the ventral nerve cords come above the endophragmal shelf through the last median aperture in the latter. They pass over the median arch formed by the mesophragma of the 12th endosternites and then proceed downwards and are continued as the abdominal nerve cords.

The abdominal nerve chain.—This lies above the sterna in the mid-ventral line. It thus lies with the flexor muscle bundles on either side. There are six ganglionic swellings along the chain, situated one in each of the six abdominal segments.

Each of the first five abdominal ganglia gives off the following nerves :—

(1) A pair of appendicular nerves to the appendages of its own segment.

(2) A pair of extensor nerves to the extensor muscles of the succeeding segment.

(3) A pair of ventral nerves to the accessory flexor muscles of that segment.

(4) A pair of dorsal nerves arising from the dorsal side of the ganglion and running obliquely backwards to innervate (*a*) the main flexors of its own segment, (*b*) the intertergal muscles between that segment and the succeeding segment and (*c*) the accessory flexors of its own segment.

(1) *The appendicular nerves* : These nerves arise ventro-laterally from the respective ganglia and after proceeding outwards almost at right-angles to the median axis of the body, enter the propodities of the appendages of the segments. There being no appendages on the first abdominal segment, the corresponding nerves instead of innervating the appendages proceed to the ventral thoracico-abdominal flexors.

(2) *The extensor nerves* : These also originate from the ventro-lateral margin of the ganglion and after pursuing first an outward course parallel to the appendicular nerves, for a short distance, turn backwards and obliquely outwards. Proceeding farther in an upward direction alongside the main flexors, they innervate the dorsal extensor muscles of the succeeding segments.

(3) *The ventral flexor nerves* : At their origin they look more like nerve bands arising partially from the connectives behind the ganglia. Each of them pursues an outward course parallel to the extensor nerves and innervates the accessory flexor muscles of that segment.

(4) *The dorsal nerves* : Each of them proceeds obliquely outwards and backwards between the main flexor muscles of its segment and that of the succeeding one, and branches into three. The distal branch innervates the main flexor of its side in that segment, the proximal, the inter-tergal muscles of its side between that segment and the next, while the middle innervates the accessory flexor of its side in that segment.

The sixth abdominal ganglion.—The sixth abdominal ganglion is larger than the others and can be assumed to be formed by the fusion of the paired ganglia of the sixth segment with others of the post-abdominal region. This ganglion gives off the following nerves:—

(1) A pair of nerves originates in front from the ventro-lateral margin of the ganglion. Each of them proceeds backwards and outwards to innervate the exopodite of the last abdominal appendage of its side.

(2) A pair of nerves originates ventro-laterally from the anterior part of the ganglion behind the nerves mentioned above. Each of them proceeds backwards and outwards and innervates the muscles of the endopodite of the last abdominal appendage of its side.

These two pairs of nerves correspond to the appendicular nerves of that segment.

(3) A pair of nerves originates from the anterior part of the dorsal margin of the ganglion. Each of these pursues a backward and outward course and innervates the last main flexor of the abdomen of its side.

(4) A pair of nerves originating close behind the origin of the appendicular nerves, runs backwards and outwards and each of them then branches into three. The outermost of these, branches several times and innervates the extensor muscles of the telson, whereas the middle and the inner branches innervate the flexors of the uropods.

(5) A pair of considerably thick nerves originates very close to each other from the posterior region of the ganglion. Each of these pursues a backward and slightly outward course and innervates the flexors of the telson of its side.

(6) Apart from the nerves described above the sixth abdominal ganglion gives off a pair of slender visceral nerves which arise from the posterior part of the ganglion. These may be called the posterior visceral nerves. Each of these proceeds downwards for a short distance and running along one side of the intestine, innervates its posterior region. The two nerves are connected by a small transverse cord. From this cord a number of nerves are given off to the last part of the intestine close to the anus.

The Cordal nerves. —There are four pairs of very slender nerves originating along the cords between the first and second, second and third, third and fourth, and fourth and fifth abdominal ganglia. Each of these nerves pursues an outward course and innervates the ventral superficial flexor of its side in that segment.

The Main Visceral Nervous System

The main visceral system consists of four ganglia from which issue nerves which are associated with the œsophagus and the stomach. The following are the chief parts of the main visceral system (Fig. 2).

The anterior visceral nerve.—This is a slender nerve which originates from the posterior aspect of the brain between the two circum-œsophageal commissures. It runs backwards up to the depression where the œsophagus joins the cardiac stomach. Farther on it runs to the roof of the cardiac stomach and after reaching the depression between the cardiac and pyloric stomachs divides into two. Each one of these branches proceeds obliquely backwards and downwards to the floor of the pyloric stomach where it ramifies. It also sends a branch—the hepatic nerve to the liver. Two ganglia are present along the course of the visceral nerve in the depression between the œsophagus and the cardiac stomach. One of these is posterior and dorsal in position and it is connected to the ganglia on the circum-œsophageal commissure on each side by a thin pair of nerves. This connection thus formed between the two circum-œsophageal commissures through the

intervention of the cords on the visceral nerve forms a supra-œsophageal connective. It may be termed the first anterior œsophageal connective.

The other ganglion situated on the anterior visceral nerve is anterior and ventral and it is connected by a pair of thin nerves on each side to the œsophageal nerves, of the respective side. These nerves form the second anterior œsophageal connective.

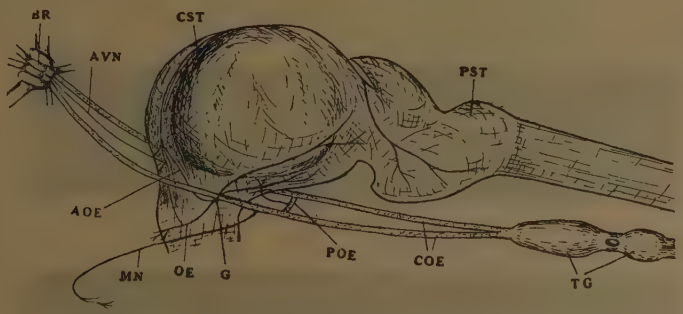

Fig. 2. The Anterior Visceral System and Associated Nerves of Panulirus

AOE, anterior œsophageal connective ; AVN, anterior visceral nerve ; BR, brain ; G, ganglion on the circum-œsophageal commissure ; COE, circum-œsophageal commissure ; CST, cardiac stomach ; MN, mandibular nerve ; OE, œsophageal nerve ; POE, posterior œsophageal connective ; PST, pyloric stomach ; TG, thoracic ganglia.

The Viscereal nerves arising from the circum-œsophageal cords.—It was mentioned in connection with the circum-œsophageal commissures that there is a ganglion situated along each of these cords. Three nerves originate from each of these ganglia and they are disposed in the following manner. The most ventral of the nerves, which originates from the ganglion runs obliquely forwards and inwards for a short distance towards the front wall of the œsophagus and then branches there. This is the œsophageal nerve. The other two nerves proceed dorsally and innervate the ventral and lateral walls of the cardiac stomach.

A delicate short nerve which arises from the posterior œsophageal connective also innervates the floor of the cardiac stomach.

The Innervation of the Heart in Panulirus polyphagus

The central nervous system of *Panulirus polyphagus* consists of a ventral nerve cord with two ganglionic enlargements in the thorax. Of these, the anterior enlargement gives off six pairs of thick appendicular nerves and five pairs of thin nerves which innervate the extensor muscles. The former arise from the ventro-lateral aspect of the ganglionic mass, whereas the latter arise from its dorsal

margin. After leaving the ganglionic mass the nerves to the extensor muscles—the origins of which are somewhat between those of the five pairs of appendicular nerves—proceed dorsally to reach the thick extensor muscles.

In order to find out from which of these thin nerves the cardiac branches arise, they were individually stimulated at the points of their origin. The behaviour of the heart on stimulation was recorded by a cardiac attachment placed against a revolving drum covered with kymographic paper. The stimulation of the first and second pair of thin nerves resulted in the inhibition of the heart (Fig. 3 A, B).

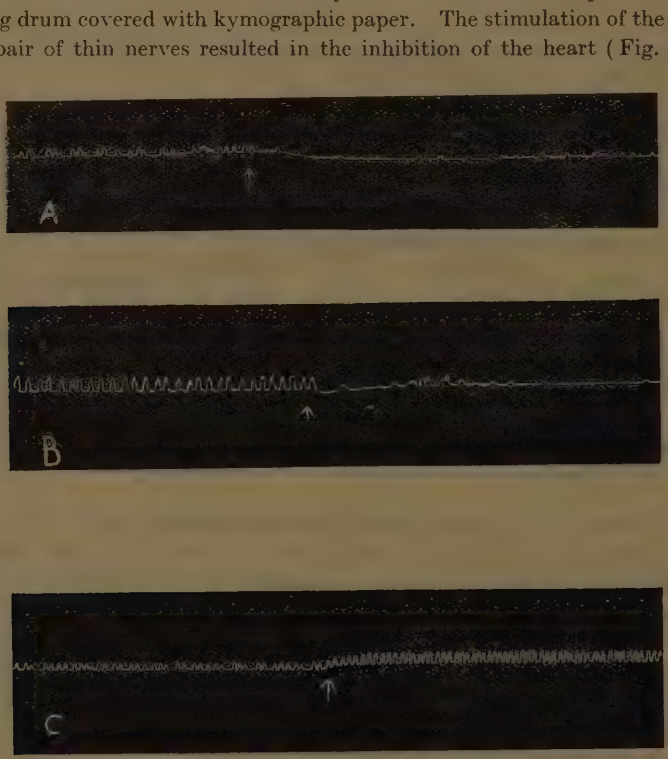

Fig. 3.A. The effect of stimulation of the 1st pair of dorsal thin nerves arising from the anterior thoracic ganglionic mass; B, the effect of stimulation of the 2nd pair of the same; C, the effect of stimulation of the third pair of the same.

The stimulation of the third pair of these nerves resulted in accelerating the beat of the heart (Fig. 3.C). On the other hand the stimulation of the appendicular nerves showed no response in the heart.

Carlson (1906) and others have shown that the decapod crustacean heart is supplied with both accelerators and inhibitors from the ventral thoracic ganglion. The results obtained here confirm their findings.

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IS THE OCCURRENCE OF DORSAL AND VENTRAL NERVES ARISING FROM THE SEGMENTAL GANGLIA IN ANNELIDS AND ARTHROPODS A FEATURE ASSOCIATED WITH METAMERISM?

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THE segmental nerves of vertebrates originate by dorsal and ventral roots. In amphioxus the corresponding roots do not meet. These facts are well known. The fact that some morphologists have recorded in crustaceans two series of nerves, one series arising from the dorsal part of the thoracic ganglion and the other from the ventro-lateral part of the cord, is not equally well known. As early as 1896 Conant and Clark recorded in the crab *Callinectes hastatus* the presence of two pairs of excitatory nerves arising from the dorsal side of the thoracic ganglion. Carlson (1906), Alexandrowicz (1932) and Smith (1947) seem to have noted a similar condition.

While investigating the nervous system of *Panulirus polyphagus*, it was noted that in addition to the normal segmental nerves which originate from the ventro-lateral region of the ganglion others taking their origin from its dorsal side also existed. The latter innervate the muscles overlying the cord and are motor in function. Some of them also contain fibres to the heart. An attempt was made to see if such dorsal nerves did exist in Nereis, Scolopendra and Cockroach and the expectations were fulfilled.

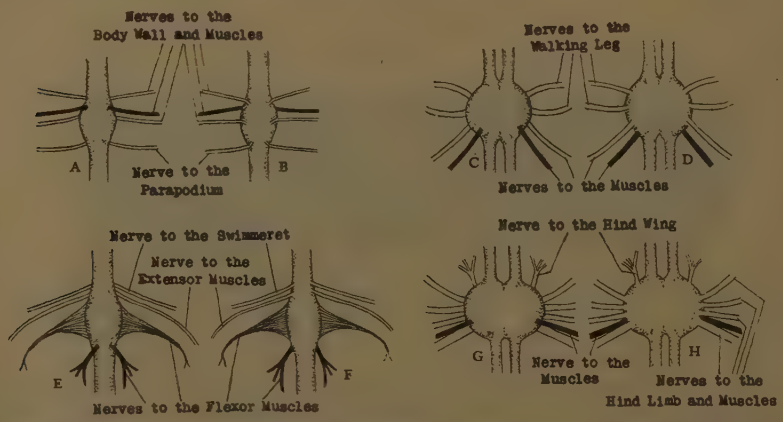

Fig. 1. The segmental ganglia, and the associated nerves in some Annelids and Arthropods.
A—Dorsal view of the fifth segmental ganglion of Nereis. B—Ventral view of the same.
C—Dorsal view of the segmental ganglion of Scolopendra. D—Ventral view of the same.
E—Dorsal view of the third abdominal ganglion of Lobster. F—Ventral view of the same.
G—Dorsal view of the third thoracic ganglion of Cockroach. H—Ventral view of the same.
Ventro-lateral nerves indicated by lines and the dorsal ones solid black.

In Nereis from a typical segmental ganglion three pairs of nerves originate ventro-lateral, and one pair dorsal. The former innervate the parapodia and the body wall, while the latter proceeds to the muscles (Fig. 1. A, B).

In Scolopendra also three pairs of nerves originate ventro-lateral and one pair dorsal. Of these, the former innervate the limbs and the body wall while the latter goes to the muscles (Fig. 1. C, D).

In the lobster Panulirus there are four pairs, arising from a typical abdominal ganglion three originating ventro-lateral and one dorsal. The former go to the appendages and some muscles and the latter goes to the flexor muscles (Fig. 1. E, F).

In the cockroach Periplaneta also a condition resembling what is met with in the above animals exists. In addition to the wing nerves, five pairs of nerves originate from the third thoracic ganglion. Of these one pair is dorsal, while the others are ventro-lateral. The dorsal pair goes to the muscles, while the others innervate the appendages and some muscles, (Fig. 1. G, H).

Discussion

In all the animals dealt with here it is perhaps not without significance that one pair of nerves originates from the dorsal side of the segmental ganglion and that this pair is exclusively motor, while the others are mixed containing both sensory and motor fibres. In the vertebrate a segmental nerve arises by two roots, one dorsal and the other ventral, the former sensory and the latter motor. In their cranial nerves, however, the dorsal nerves are mixed while the ventral ones are motor. A parallelism between the vertebrate segmental nerves and the annelidan and arthropodan ones is thus obvious, provided the body in the latter groups is turned upside down. In that case the ventro-lateral mixed nerves of annelids and arthropods will correspond to the mixed dorsal cranial nerves of vertebrates, and the dorsal motor nerves of the former to the ventral motor ones of the latter. In this connection the various theories put forward to derive the vertebrates from either the annelid or the arthropod may be remembered. The essential feature of such theories was the reversal of the upper and lower sides so as to bring the nerve cord of the annelid or the arthropod to the dorsal side. The protagonists of these theories did not stop with comparing the nervous system alone; they thought—even the vascular system in the two groups could be compared in important respects. It is not necessary here to go into the arguments advanced in favour and against these theories but it may be mentioned that none of them has been found satisfactory in most respects. All the same, the parallelism between the vertebrate and the annelid-arthropod stem cannot be easily ignored. In this paper it has been shown that it could be extended to the segmental nerves also. Without going into the relative merits of the various theories of vertebrate origin, in the light of what has been described here, one is led to arrive at the irresistible conclusion that the isolation of nerves into dorsal and ventral ones of which one set is exclusively motor is a feature some way associated with metamerism.

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ON THE CRANIAL OSTEOLOGY OF *UROMASTIX HARDWICKII* (*GRAY*)

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WHILE examining the skull of *Uromastix hardwickii* for teaching purposes, it was noted that it differs in certain respects from that of *Uromastix ægyptia* as described by El-Toubi. It was therefore thought desirable to record those differences and at the same time give a brief description of the skull of this species.

General features of the Skull

The skull of *Uromastix hardwickii* is typically lacertilian and strongly ossified like other agamid skulls. The orbit is completely surrounded by bone as in Calotes (Iyer, '43) unlike in certain other lizards like Varanus (Bahl,' 39) and Hemidactylus (Mahendra, '49). The infratemporal fossa is small and is bridged over by an arch formed by the squamosal, jugal and post-orbital. The premaxilla is single. The nasals are paired as in Sphenodon (Gunther, 1867), Lacerta (Parker, 1879), Tupinambis (Reese, '23), Scincus (El-Toubi, '38), Calotes (Iyer, '43) and Hemidactylus (Mahendra, '49). The frontals are unpaired as in Calotes (Iyer '43) and unlike as in Lacerta (Parker, 1879) and Varanus (Bahl, '39). The parietals are also unpaired as in most lizards. The distinct lacrimal present in Varanus (Pahl, '39), Lacerta (Parker, 1879), Tupinambis (Reese, '23), and Scincus (El-Toubi, '38), is absent in Draco (De Beer, '37), Calotes, Hemidactylus and in this genus. The snout is short and arched upwards. Consequently the nostrils are placed in front and not on the top of the skull. The orbit is considerably large. The pineal aperture occurs at the junction between the frontal and parietal in the median line, The inter-orbital septum is highly membraneous. The basisphenoidal rostrum is clearly marked, pointed and narrow. The teeth on the maxilla slant inwards and are united to form a single block. The cutting edge is inner and lateral on the upper jaw and outer and lateral on the lower.

Dorsal Aspect of the Skull (Fig. 1)

The premaxilla is a narrow single bone wedged in between the nasals behind and the maxilla in front. It is vertically placed as the skull in that region is arched and it extends posteriorly as the *processus nasalis*, but does not reach the frontals as is the case in *Uromastix ægyptia*. The fontanelle present in many agamids (Siebenrock, 1895) is covered over by an anterior extension of the frontal, in the

Indian species but in the Egyptian form it is obliterated by the posterior extremity of the *precessus nasalis* of the premaxilla (El-Toubi, '45) which was wrongly

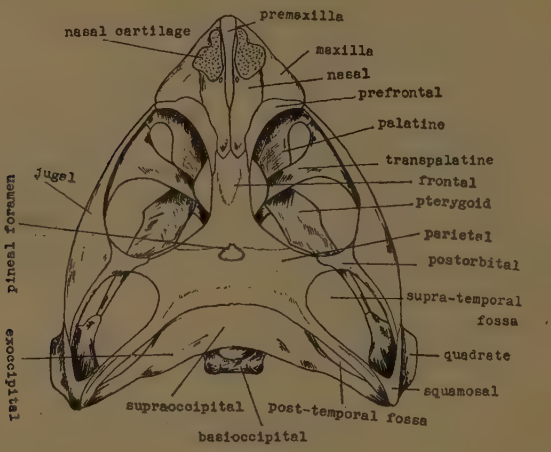

Fig. 1. Dorsal view of th skull of *Uromastix hardwickii*.

described by Beddard ('05 b) as the mesethmoid. The extent of the process of the frontal lying between the nasal and prefrontal varies in both. From a comparison of less and more ossified skulls of the Indian form, it has been found that in the former, the process extends to the nasal vacuity and in the latter its anterior portion is covered by the nasal. The variations thus seem to be the result of age. The nasals are pawed. The nasal opening is bounded by the nasal on the inner margin, the prefrontal behind and the premaxilla below. All round the nasal opening there is less ossification, so much so that in well-prepared skulls the membraneous portion around this opening is clearly seen as such. The orbital fossa is bounded on the inside by the single frontal, in front by the prefrontal, behind by the parietal, postorbital and the jugal, and on the outside by the jugal only. The parietal foramen lies on the fronto-parietal suture and gets drawn out into a large vacuity on the parietal. In most *Uromastix ægyptia* skulls examined by El-Toubi the parietal foramen lies on the fronto-parietal suture but in a few he found it lying completely inside the frontal. In some lizards like *Chalcides guentheri* (Hass, '36) and *Scincus scincus* (El-Toubi, '38) the parietal foramen occupies a position in the anterior part of the parietal bone. The degenerate postfrontal described by Beddard ('05 b) in *Uromastix ægyptia* is absent in the Indian species. Some specimens of *Uromastix ægyptia* are without the postfrontal on one side and some completely devoid of them (El-Toubi '45). The absence of the postfrontal in Chlamydosaurus, Amphibolurus and Physignathus was recorded by Beddard ('05a). This indicates that the tendency towards the disappearance of this bone in *Uromastix ægyptia* is a *fait accompli* in *Uromastix hardwickii*.

Lateral Aspect of the Skull (Fig. 2)

The supratemporal fossa is bounded by the postorbital and the parietal in front, by the squamosal behind, and by the postorbital outside and the parietal

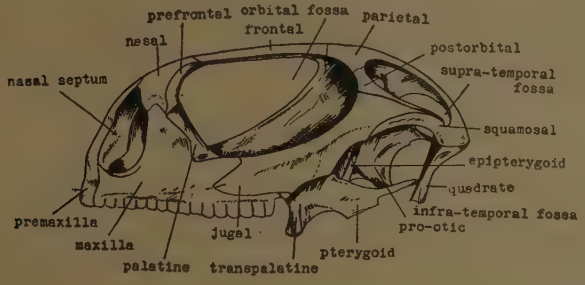

Fig. 2. Lateral view of the skull of *Uromastix hardwickii*.

inside. Variation in the formation of the supratemporal arcade is met with in the Indian species as in the Egyptian one. The postorbital in some meets the squamosal by means of an extension, thus forming the lateral boundary of the supratemporal fossa and as such prevents the jugal from sharing in bounding the lateral wall of the fossa, while in others such an extension is not visible in the dorsal view. From an examination of several skulls it has been found that the variation is a matter of age, since in the older more ossified specimens the extension is well marked, but not so in the younger less ossified ones. The infratemporal fossa is bounded by the jugal and the squamosal above and the quadrate behind, while the lower boundary is unbridged. The post-temporal fossa is bounded by the extension of the parietal above, supraoccipital inside and the exterior arm of the exoccipital and the opistho-otic below.

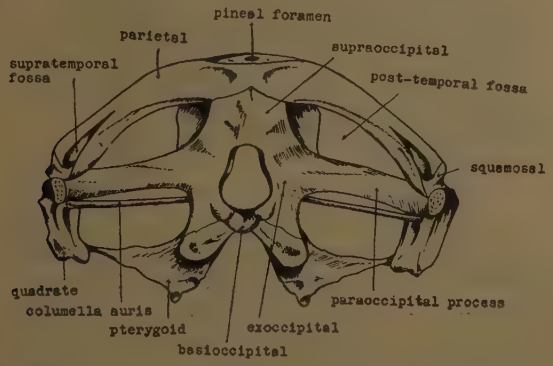

Fig. 3. Posterior view of the skull of *Uromastix hardwickii*.

Posterior Aspect of the Skull. (Fig. 3)

The *foramen magnum* is surrounded by the supraoccipital bone above, the exoccipital at the sides, and the basioccipital below. The single condyle is formed by the basioccipital. The exterior arm of the exocciptal, the paraoccipital process, meets the squamosal and the top of the quadrate in the postero-lateral corner of the skull. The quadrate is a flattened bone directed vertically, with a flat face in front and behind. Its upper attachment is with the squamosal, while by its lower end it articulates with the lower jaw.

Ventral Aspect of the Skull (Fig. 4)

On the floor of the skull, the basioccipital forms a junction with the basisphenoid in front. The basisphenoidal processes diverge outwards in front to meet the inner corners of the slightly oblique pterygoids. The basisphenoidal rostrum slants forwards and joins the frontal on the ventral side. The pterygoid is flat in its front half, but vertically slanting behind where it is joined to the quadrate by a narrow process. Near about its junction with the basisphenoidal process stands the epipterygoid directed obliquely backwards and upwards meeting the roof of the skull at the junction between the parietal and the pro-otic. The pro-otic is ossified and forms the front part of the auditory capsule. The hind part is formed by the opistho-otic. A long slender *columella auris* is attached to the tympanum and the quadrate. The transpalatine is a rather vertically-disposed bone in its inner portion which meets the middle portion of the pterygoid in the

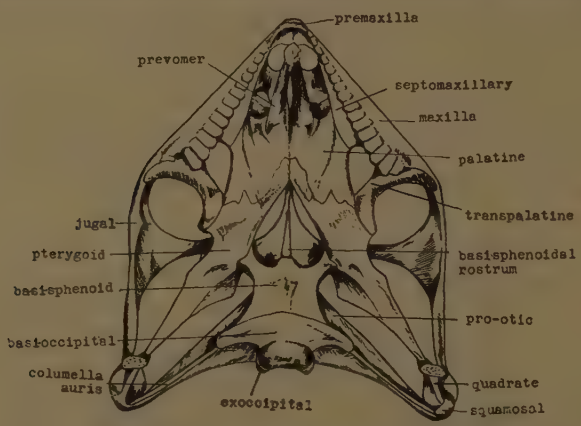

Fig. 4. Ventral view of the skull of *Uromastix hardwickii*.

floor of the skull. Its outer portion which is horizontal, joins the maxilla just behind the last tooth. The palatine is placed anterior and lateral to the pterygoid and it meets the prevomer on the inner side in front, and the maxilla on the outer. In this meeting two fossæ are left, one behind and the other in front. The hind one is bounded on the outer side by the maxilla, behind by the transpalatine, on the inner side and front by the palatine and maxilla. The front fossa is bounded

by the maxilla on the outer and front sides, by the palatine behind and the prevomer on the inner side. A small vacuity said to be existing between the palatopterygoid bones in the Egyptian form does not occur in the Indian species. The fully developed quadrate process of the pterygoid in *Uromastix ægyptia* reaches the quadrate, while it does not quite do so in *Uromastix hardwickii* (Saksena, '42). The prevomers are two vertically oblique bones diverging on the ventral aspect but uniting on the dorsal where it joins the ventral wall of the cranium below the nasal region. A small vacuity exists between the prevomers but it is restricted in the local form. The continuation of the narrow cavity between the prevomers with that between the palatines and pterygoids observed in the Egyptian form, is absent in *U. hardwickii*. Beddard ('05b) laid great stress on the anterior extension of the pterygoid as far as the prevomers in *Uromastix spinipes* (*U. ægyptia*) although they did not actually touch the prevomers. He compared this feature of the pterygoids in *Uromastix ægyptia* to that in Sphenodon in which the pterygoids actually meet the prevomers (Howes and Sinnerton '01). In *Uromastix hardwickii* the extensions of the pterygoids go even farther than in the Egyptian form, so much so that on a casual observation one is tempted to think that they are actually in contact with the posterior ends of the prevomers. Saksena ('42) has clearly shown that there is no parallelism between Sphenodon and Uromastix, in this respect, since it is a process of the palatine immediately in front of the pterygo-palatine suture which he calls the prevomerine process, which meets the prevomer. The pterygo-vomerine contact in Sphenodon is usually regarded as a primitive feature but its real morphological significance is doubtful.

Lower Jaw (Figs. 5 and 6)

The lower jaw is flattened laterally and is formed by the usual six bones including the splint-like splenial which is small or absent in agamids (Camp '23) and in

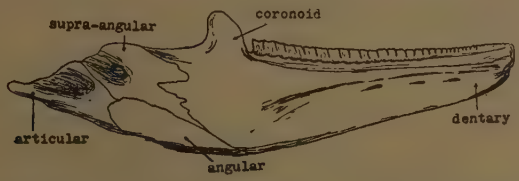

Fig. 5. Outer view of the lower jaw of *Uromastix hardwickii*.

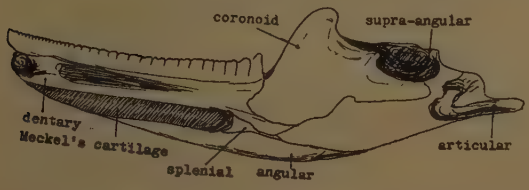

Fig. 6. Inner view of the lower jaw of *Uromastix hardwickii*.

addition the slender persistent Meckel's cartilage. The two dentaries are united in front by separable symphysis. The dentary bears a row of acrodont and homodont teeth joined together as in the upper jaw. The post-articular process of the dentary behind the articular cavity which is well marked in Calotes and Varanus (Iyer '43) is reduced in Uromastix.

Summary

The skull and lower jaw of *Uromastix hardwickii* are briefly described and points of contrast with those of *Uromastix ægyptia* are emphasised. The following are the more important amongst them.

(1) The *processus nasalis* in *U. hardwickii* does not reach the frontal.

(2) The fontanelle is covered by an anterior extension of the frontal and not by the posterior extremity of the *processus nasalis* as in *U. ægyptia*.

(3) The postfrontals are absent.

(4) The parietal foramen is on the fronto-parietal suture.

(5) The continuation of the narrow vacuity between the prevomers with that between the palatines and pterygoids observed in the Egyptian form, is absent in *U. hardwickii*.

(6) The fully developed quadrate process of the pterygoid in *U. ægyptia* reaches the quadrate but does not quite do so in *U. hardwickii*.

(7) The pterygoids in *U. hardwickii* extend much more forwards towards the prevomers than in *U. ægyptia* but do not actually meet them.

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RESPIRATORY MECHANISM IN THE CHELONIA

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THE respiratory mechanism in the Chelonia has received considerable attention from zoologists because of the physiological problems involved as a result of the unique skeletal adaptations that have taken place in these animals. The rigid shell has made all free movements of the body wall impossible and consequently the respiratory movements characteristic of other amniote groups are absent. The structural modifications in the Chelonia naturally have created an intriguing situation for zoologists, since the mechanism of respiration in them cannot be explained on the same lines as for the other reptiles.

Malpighi, Cuvier, Agassiz (1857) and even some of the recent authors like Noble and Noble (1940) and Walters (1949) have suggested that the throat movements as in the frogs are the effective part of the respiratory mechanism. Mitchell and Morehouse (1863) have laid great emphasis on the part played by the musculature of the glottis in respiration but much attention had not been paid to their work by later investigators. Wolf (1933) proposed that the movements of the neck and the limbs indirectly served the purpose of the body-wall movements necessary for primary pulmonary action. Ludicke (1936) studied the rôle of throat movements and observed that aquatic forms like *Emys orbicularis*, swallow air but terrestrial ones like *Testudo gracea* do not do so on account of their inability to secure an airtight closure of the mouth.

McCutcheon (1943) conducted some interesting experiments to study the respiratory mechanism of turtles and brought forth certain significant findings. He observed that the contraction of the anterior and posterior flank cavity muscles, the *serratus magnus* and the *oblique abdominis* causes increase in the volume of the body cavity. The decrease in pressure caused there, forces the lungs to expand, and air from outside rushes into the lungs, bringing about inspiration. On the other hand when the *diaphragmaticus* and the *transverse abdominis* contract, pressure is exerted on the lungs resulting in expiration. He refers to the posterior flank cavity muscle, the *oblique abdominis* as the more important agent in the inspiratory mechanism. He is also of the opinion that the throat movements cannot pump air into the lungs, because the force created thereby is insufficient to open up the glottis. Moreover, he points out that evidence is available to show that the throat movements are associated with olfaction.

Recently we (1954) pointed out the occurrence of two striated muscle sheaths, in the common Indian pond turtle *Lissemys punctata granosa* around the lungs. The inner of the two is in the lung tissue and the other occurs as a loose

outer covering. When they contract expiration is brought about and when they relax inspiration takes place. Thus our findings in that animal were that these

Fig. 1. A *camera lucida* sketch of a portion of a transverse section of the lung of *Lissemys punctata*.

Al., Alveolus. A.T., Areolar tissue. Bv., Blood vessel. C.T., Connective tissue. E. Epimysium. F.T., Fibrous tissue. Sm.M., Smooth muscle. St.M. Striated muscle (cut transversely.

two muscles enabled the lungs to function as a pair of bellows. Further, we noted that the inner striated muscular layer occurring on the wall of the lungs was innervated by the intercostal nerves. We have therefore suggested that it was homologous to the intercostal muscles of the other reptiles.

We have now extended our study to three other Chelonians viz., *Lissemys punctata typica*, *Lissemys punctata scutata* and *Geomyda trijuga*. On cursory exami-

Fig. 2. A *camera lucida* sketch of a portion of a transverse section of the lung of *Geomyda trijuga*.

C., Connective tissue. St.M., Striated muscle (cut longitudinally). (Other lettering same as in Fig. 1).

nation a pronounced striated layer was evident on the lung wall in the first two but not in the last. Microtome sections, however, revealed its presence in *Geomyda trijuga* also (Fig. 2). The occurrence of a striated muscle on the lung wall in the turtle Lissemys (Fig. 1) and in the semi-aquatic chelonian Geomyda suggests that such might be universally present in the Chelonians. A detailed study of the lungs of some other turtles and tortoises, along with that of some members of the other reptilian groups for comparison, becomes now essential and such is in progress.

Summary

In the three pond turtles *Lissemys punctata granosa*, *Lissemys punctata typica* and *Lissemys punctata scutata* and the semi-aquatic chelonian *Geomyda trijuga* it is found that the lung wall possesses an outer striated muscle layer. It is suggested that it helps in the contraction and relaxation of the lungs which cause expiration and inspiration respectively and that it may be universally present in all Chelonians.

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PHYSIOLOGY OF DIGESTION IN *MELANIA CRENULATA*

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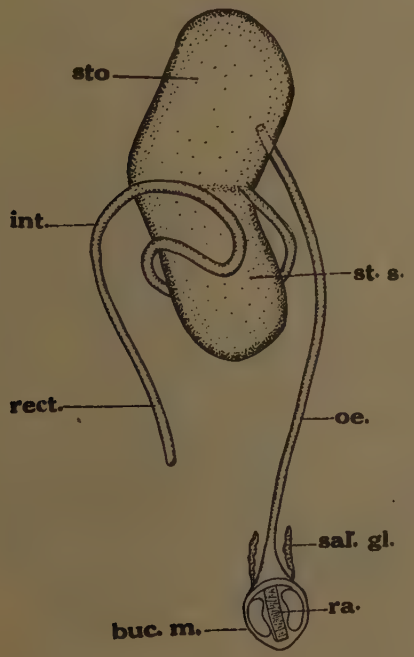
In a previous paper (2) the author gave an account of the physiology of feeding and digestion in *Pila virens*. The present contribution deals with the physiology of digestion in another Prosobranch, *Melania crenulata* which lives in tidal-rivers. A crystalline style is present in this species as reported by Seshaiya (1929).

Material and Methods

Specimens of *Melania crenulata* were collected from the Coleroon river, South India, five miles from the University campus. They were kept for observation in sufficient numbers in the aquaria in the laboratory.



Text. Fig. 1. Alimentary canal of *Melania crenulata*.

buc.m. buccal mass ; *int.* intestine ; *oe.* oesophagus ; *ra.* radula ; *rect.* rectum; *sal.gl.* salivary gland ; *sto.* stomach ; *st.s.* style sac.

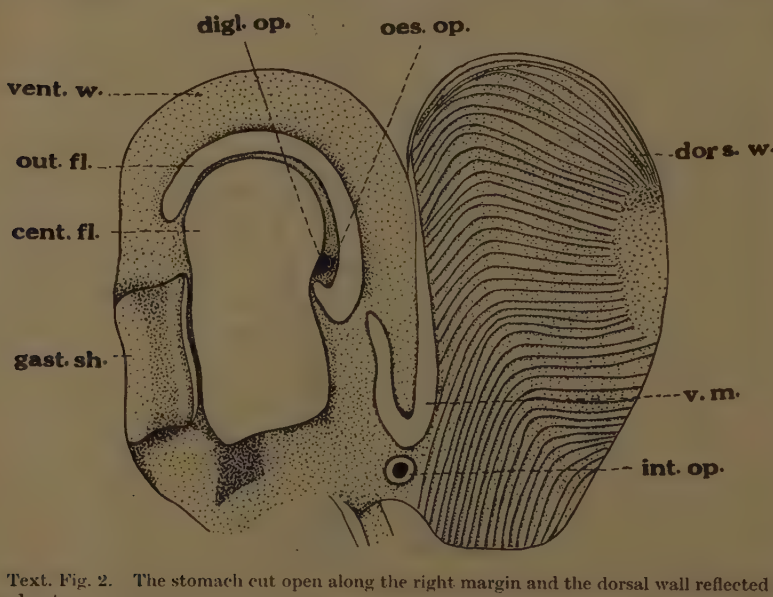
For the study of the feeding currents, the living stomach was isolated and opened in physiological saline solution and the course of the currents followed with the aid of carmine particles and carborundum powder of different grades, the observations being made under the stereo-binocular microscope.

For experimental feeding the animals were previously starved. Indian ink, boiled rice, iron saccharate and olive-oil stained with Sudan III were used as experimental diets.

The technique employed for feeding experiments and for histological work was the same as for the study of *Pila*.

The Alimentary Canal

The structure of the alimentary canal of the Tiariidæ (Melaniidæ) has been examined by Moore (1898), Graham (1939) and Seshaiya (1935). The latter author has given a complete account of the alimentary canal of *Paludomus tanschaurica*. The more important features of the alimentary canal of *Melania*, which are relevant for the present investigation are as follows :—(1) The buccal mass is very small and the radula also small. (2) A pair of salivary glands open into the buccal cavity. (3) The œsophagus measures about 3·4 cm. and opens into the stomach proper. (4) The stomach is distinguishable into an anterior thimble-shaped style-sac and a



Text. Fig. 2. The stomach cut open along the right margin and the dorsal wall reflected and spread out.

cent. fl. central fold ; *digl. op.* opening of the digestive diverticula; *dors.w.* dorsal wall ; *gast. sh.* gastric shield; *int.op.* intestinal opening ; *oes.op.* œsophageal opening ; *out.fl.* outer fold ; *st.* style ; *v.m.* V-shaped part of the marginal fold.

posterior gastric chamber or stomach proper with digestive diverticula. (5) The intestine which measures about 4·1 mm., commences at the junction of the stomach and style-sac, and looping under the style-sac runs forward to open at the anus.

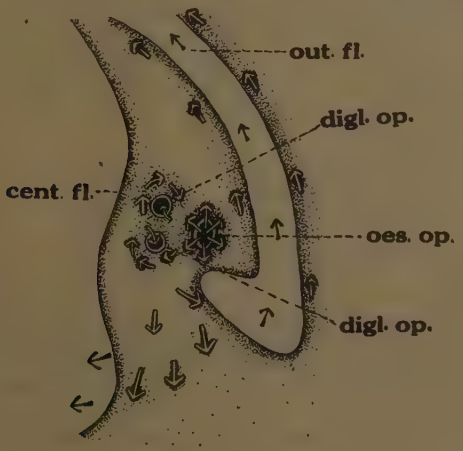
The stomach of *Melania* resembles very closely that of *Paludomus* described by Seshaiya (1929) who, however, did not study the feeding currents.

As in *Paludomus*, the gastric chamber, or stomach proper, of *Melania* has a slight protrusion antero-laterally, close to the intestinal opening. The internal structure of the stomach is shown in Text Fig. 2. The folds also resemble those in *Paludomus*. There is a broad central or inner fold. Separated from this by a narrow gutter is a narrow ridge, the outer fold. In addition to these, there is a lateral fold which is thin and extends along the left margin of the ventral wall of the stomach. Anteriorly it has a V-shaped termination. The openings of the digestive diverticula are situated between the inner and outer folds. The intestinal opening is more anteriorly situated, lying immediately in front of the V-shaped termination of the lateral fold. The gastric shield is situated on the right side of the lower part of the central fold.

The histology of the stomach of *Melania* resembles closely that of *Paludomus*. The greater part of the stomach is ciliated except in the region of gastric shield, and in a small area in the dorsal wall. The cells on the folds have prominent cilia. Goblet-shaped mucus cells are abundant on the folds especially the central fold. Muscle fibres are absent in the stomach wall of *Melania* in contrast to the stomach of prosobranchs like *Pila*.

The style-sac and crystalline style are like those of *Paludomus* and have been described by Seshaiya (1929).

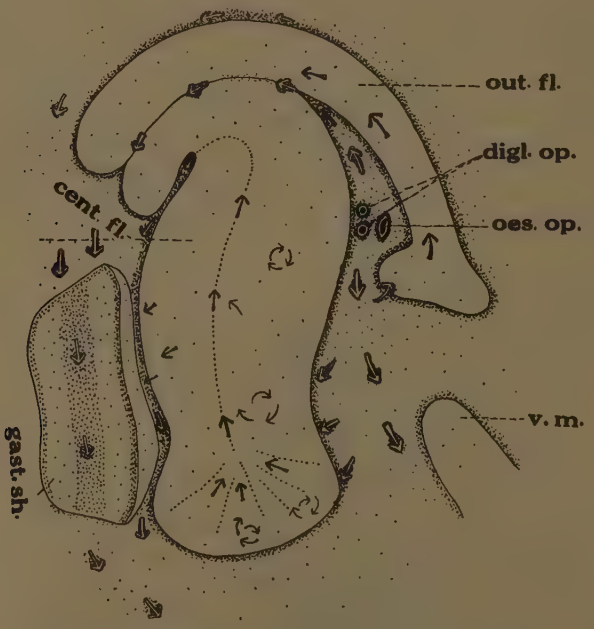
The Ciliary Currents in the Stomach



Text Fig. 3. The currents in the region between the central and outer fold. Lettering as in Text Fig. 2. The arrows indicate the direction of the food currents.

The ciliation of the stomach is much more extensive than in *Pila*, the cilia being absent only in the region of the gastric shield as mentioned already. In accordance with this extensive ciliation, conspicuous ciliary currents occur nearly all over the lining of the stomach.

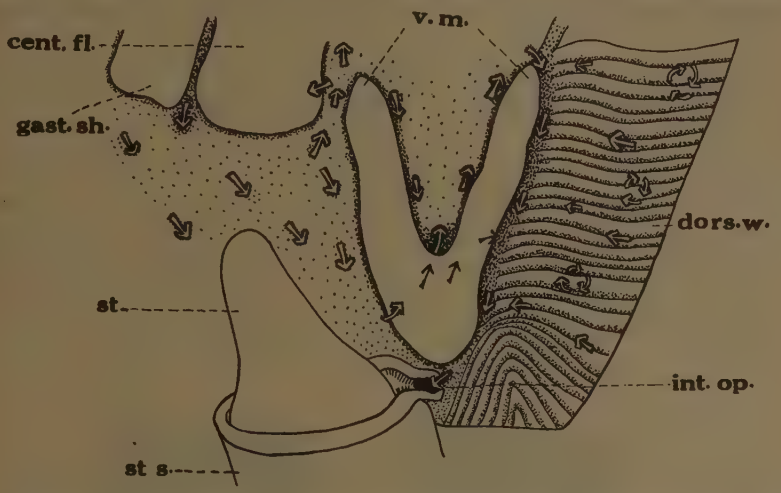
Powdered carmine and carborundum of different grades were employed for demonstrating ciliary currents. When either of these materials is dropped in the region of the œsophageal opening, whirlpools of ciliary currents are noticed. The lighter and finer particles are directed to the opening of the digestive gland, which is close to the œsophageal opening. The coarse and heavier particles are rejected from this region and ultimately directed to the region of the gastric shield (Text Figs. 3 and 4). This diversion is brought about mainly by the ciliary currents between the outer and inner folds of the ventral wall of the stomach. In the region of the gastric shield the food particles are subjected to the mechanical as well as



Text. Fig. 4. The folds and the food sorting mechanism. Lettering as in Text Fig. 2.

chemical action of the crystalline style. The mechanical effect of the rotation of the style produces a constant stirring of the stomach contents. The food particles, as stated above, are directed from the grooves between the folds to the region of the gastric shield and thence travel to the openings of the digestive diverticula, where the finer partly-digested particles are admitted in and thus pass into the digestive diverticula. The coarser particles ultimately reach the intestinal opening (Text Fig. 5). The ciliary movements on the transverse ridges of the dorsal wall

and the V-shaped fold constitute the mechanism for the sorting of food particles. The passage of the lighter particles from the region of the gastric shield to the openings of the digestive diverticula is effected by the more or less transverse currents

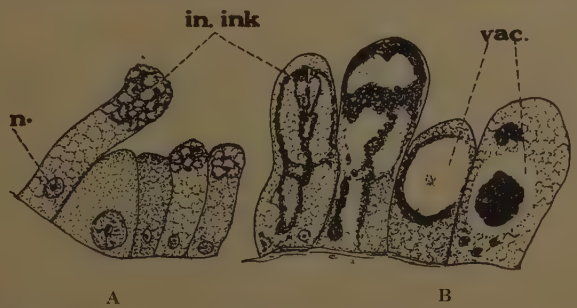


Text Fig. 5. The currents in the anterior part of the stomach. Lettering as in Text Fig. 2.

of the dorsal wall of the stomach. The coarse particles accumulate in the left corner of the anterior end of the stomach, where the cavity is deeper and rather pouch-like and in close proximity to the intestinal opening.

Feeding Experiments

Indian ink feeding :—Powdered Indian ink was mixed with filamentous algæ to facilitate ingestion by the animal. The Indian ink particles as revealed by sections pass into the stomach and reach the tubules of the digestive gland. The cells of the tubules show various stages of ingestion of Indian ink particles (Text Fig. 6 A and B). The free margins of the cells develop an irregular outline and project



Text Figs. 6 A & B. Stages in the absorption and excretion of Indian ink. *in. ink.* Indian ink ; *vac.* vacuole.

into the lumen of the tubule. In the early stages of feeding the particles are found inside the cells close to the margin. Later, the particles are distributed in a more or less reticulated pattern. After feeding for a few days, *i.e.* in the later stages of absorption, the particles accumulate in the central and proximal part of the cell as spherical masses. In still later stages the Indian ink is concentrated in the large excretory spherules enclosed in the vacuoles (Text Fig. 6) in the cells of the crypts of the tubule. Almost all the cells of the tubules show evidence of ingestion of Indian ink. After prolonged feeding there is rejection of Indian ink from the so-called excretory cells (Pl. I) and it appears in the rectum and fæcal pellets.

Iron saccharate feeding :—When *Melania* is fed on this substance the cells of the digestive gland showed large amount of iron in varied stages of absorption. No iron was found in the intracellular portions of the digestive gland as is noticed in the case of bivalves fed on iron saccharate. In the early stages of absorption the iron saccharate is found in the distal ends of the cells of the digestive gland. In later stages the ingested particles appear in small masses enclosed in vacuoles and in still later stages, iron can be detected in the excretory spherules.

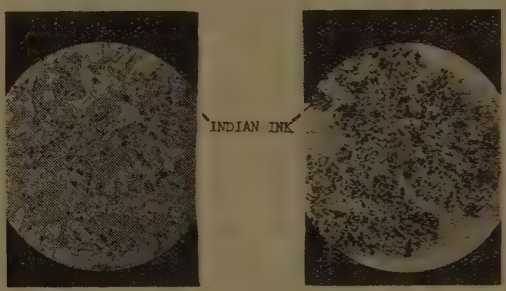


PLATE I

Photomicrographs of the sections of the digestive gland of ink fed *Melania* showing absorption and excretion of Indian ink.

The Digestive Enzymes

The digestive enzymes in *Melania* are located in the salivary glands, crystalline style and digestive gland. The cells of the salivary gland secrete a juice which is conveyed to the buccal cavity through the ducts of the salivary glands. From the crystalline style the enzymes are released into the stomach where the free end of the style dissolves. The enzymes of the digestive gland act intracellularly. It is interesting to notice that there is both extracellular and intracellular digestion in *Melania*. The cells of digestive gland ingest the food particles and digest them within.

A strong amylase is found in the extracts of the salivary gland, digestive gland and the crystalline style. The optimum pH of this amylase was found to be

about 5·9 in all cases. Besides the amylase, a weak glycogenase is also found in the salivary gland extract. The crystalline style contains in addition to an amylase a maltase and a weak invertase. The digestive gland extract contains almost all the important carbohydrates such as amylase, maltase, cellulase, invertase, and a weak glycogenase. A lipase is present in the digestive gland extract but no protease has been detected. The optimum temperature for these enzymes ranges between 37°C and 40°C.

Discussion

Malania crenulata is a microphytophagous continuous feeder inhabiting tidal rivers, unlike *Pila* which is macrophytophagous, feeds discontinuously and inhabits ponds. These differences are reflected in the anatomy and physiology of the digestive system of the two species.

The important features of the alimentary canal of *Melania* are :—

1. The buccal mass is smaller and the radular teeth and cusps are narrower and smaller.
2. The salivary glands are very small and œsophageal pouches are absent.
3. In the stomach there is an anterior diverticulum which comprises the style-sac and contains a style. In the stomach proper there is extensive ciliation and development of prominent folds and ridges as well as a gastric shield.

The loss of œsophageal pouches is intimately associated with the characteristics found in the style-bearing forms, as Yonge (1932) has pointed out. Graham (1939) has pointed out that prosobranchs living in habitats where continuous feeding is possible, have a style and this is what we find in *Melania*. The continuous feeding associated with the presence of style in its turn is related to the absence of œsophageal pouches. Oesophageal pouches are found as Graham (1939) pointed out, in animals which have a rhythmic or discontinuous feeding, the food coming only in irregular and unpredictable hours.

The stomach of *Melania* shows devices for mixing the food particles with the style-substance and a food-sorting mechanism for diverting finer particles into the opening of the digestive diverticula.

The greater length of the intestine in *Pila*, as compared with that of *Melania* is related to the discontinuous feeding and to the much greater volume of food consumed. Thus in the case of a microphytophagous continuous feeder like *Melania* there is no necessity for a long intestine.

The differences in the mechanism of digestion in the two species *Melania* and *Pila*, like the extent of ciliary currents and the crystalline style have been men-

tioned already. The most important difference, however, between the two species is the intracellular digestion in *Melania*, which again is intimately bound up with the microphagous diet of the animal.

The biochemical aspects of digestion in the two species do not differ considerably. Both *Pila* and *Melania* are herbivorous animals and in both of them cellulase is found and is particularly powerful in *Pila* which has to digest a large amount of cellulose food extracellulary in the stomach.

The localisation of amylase in the two forms differs to some extent. This is in accordance with an amylase-containing crystalline style in *Melania*. The specificity of the amylase, however, whenever it is present is the same as indicated by the optimum pH required for its action.

A protease has not been detected as in *Pila*. How these animals meet their protein requirements is being investigated.

Summary

(1) The structure and function of the digestive system of *Melania crenulata* are described, chiefly with reference to the stomach and digestive diverticula.

(2) Ciliary currents in the stomach of the animal have been studied and feeding experiments have been carried out employing substances like Indian ink, iron saccharate, olive oil, boiled rice etc.

(3) *Melania* is a microphytophagous rhythmic feeding herbivore.

(4) In *Melania* there is *intracellular* digestion in the digestive diverticula.

(5) A style-sac with a crystalline style is present. Related to this feature is the absence of the œsophageal pouches (which are present in forms like *Pila*).

(6) The food-sorting mechanism in the stomach is described.

(7) The digestive enzymes in the salivary gland, crystalline style and digestive gland were studied. A cellulose is present localised in the digestive gland. A protease has not been detected but a lipase is present.

(8) The digestive gland is the sole organ of absorption.

Acknowledgment

I have great pleasure in expressing my thanks to Prof. R. V. Seshaiya for suggesting this line of investigation and for instruction and guidance. My thanks are also due to the Annamalai University for awarding me a research studentship during the period (1950 Sept. to 1951 Dec.) when this investigation was carried out.

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A COMPARATIVE STUDY OF THE BLOOD PLASMA OF SOME CRUSTACEANS AND MOLLUSCS

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THE characterisation of blood as a part of the "milieu interior" by the great nineteenth century physiologist Claude Bernard directed attention towards the rôle of blood in maintaining a more or less constant internal environment suitable for the metabolic activities of the organism. It has to be recognized that its present status as an evolutionary end product has been attained through progressive changes lasting for ages. Though Claude Bernard's remark was applied to the vertebrate blood in all its complexities, it applies with equal force to the fluid internal medium of all animals. But only the vertebrate blood has been studied in its various aspects, and that of the lowly-evolved groups has received scant attention. Nevertheless, it merits intensive study, more so as an internal environment, on account of the success which these groups have achieved in establishing themselves in the sea, fresh-water and land.

Amongst the substances present in the plasma, the proteins are of special significance, since they contribute materially to the physical properties of the fluid internal environment. From previous works it is found that the amount of plasma proteins in the blood shows a range of variation. This paper deals with the plasma proteins of the blood of crustaceans and molluscs in which groups it is generally blue. Determinations of total proteins as well as of albumin and globulin in the blood of a few of them have been made (Table I). For purpose of comparison total protein values available from previous literature are also summarised (Table II).

Material and Methods

Preparation of the material for analysis:—For analytical purposes blood was drawn from living specimens with the aid of a small glass syringe. It was transferred immediately into 10 c.c. centrifuge tubes coated on the inner surface with a thin dry film of potassium oxalate which served as an anti-coagulant. In the crustaceans, blood was drawn from the region of the pericardial sinus by a careful manipulation of the needle inserted through a minute pin hole made in the carapace. In Octopus, Pila, Paphia and Lamellidens, blood was drawn directly from the auricles. Many specimens of molluscs were used for getting 5 to 6 cc. of blood. In Telescopium and Aplysia blood was collected from the various sinuses. After transferring the blood into the tubes, they were carefully rotated to dissolve

the potassium oxalate and immediately stoppered to prevent evaporation. They were then centrifuged at first gently and then at a speed of 2,500 revolutions per minute. This led to the separation of corpuscles and plasma, the former being packed into a compact layer at the bottom of the tube. The plasma was then separated with the help of a pipette.

Determination of plasma proteins :—The protein estimations were carried out by the micro-Kjeldahl method as recommended by Hawk *et al.*, (1948) which is a combined adaptation of the procedures of several authors. Total proteins in the plasma were estimated by the direct nesslerization micro-Kjeldahl method of Wong (1923), making the appropriate correction for the non-protein nitrogen. The non-protein nitrogen was estimated by the micro-Kjeldahl method from the protein-free filtrate which was prepared by the method of Folin and Wu (1920). Attempts were made to determine the fibrinogen in the plasma after isolation as fibrin by digestion and direct nesslerization. The isolation of fibrinogen as fibrin was tried according to the method of Cullen and Van Slyke (1920), but the attempts were unsuccessful as the nature of the fibrin formed after the addition of 2·5% calcium chloride solution was in the form of suspension and could not be easily separated from the original solution. The amount of albumin was determined by analysis of the fluid remaining after precipitating the globulin fraction with 23% sodium sulphate. This fractionation of the protein with sodium sulphate solution was according to the procedure of Howe (1921). This method was slightly modified in that ether was used to aid in separating the albumin and globulin fractions as suggested by Kinsgsley (1940). The amount of globulin is usually found by subtracting the amount of albumin and fibrinogen from total protein. The values of globulin in crustaceans are approximate since they are inclusive of fibrinogen.

Results

Table I

Total proteins, albumin and globulin in the blood of some crustaceans and molluscs

Animal	Total protein g./100 cc. of plasma	Albumin g./100 cc. of plasma	Globulin g./100 cc. of plasma	Albumin / Globulin
Crustacea :—				
1. *Panulirus polyphagus* ...	8.044–8.768	6.178–6.635	2.123–3.733	1.76–2.59
2. *Scylla serrata*	3.23—4.25	1.78–2.39	1. 45–1.93	1. 0–1. 5
3. *Paratelphusa guerni* ...	2.206–2.83	0.457–0.6	1.748–2.28	0.23–0.26
Mollusca :—				
4. Octopus sp.	5.33–6.818	0.317–0.643	4.687–6.501	0.048–0.1
5. Aplysia sp.	3.526	1.451	2.075	0.6
6. *Telescopium telescopium*	1.256–2.845	0 .106–0.657	1.15–2.188	0.09–0.3
7. *Paphia malabarica* ...	0.123	0.011	0.112	0.098
8. *Pila globosa*	1.13—1.94	0.0277–0.282	1.1023–1.658	0.025–0.1
9. *Lamellidens lamellidens*	0.0642–0.0817	0.018	0.0462	0.38

Table II

Total protein values collected by Prosser and Florkin from previous works for some crustaceans and molluscs

Animal	Total protein values in g. 100 cc. of plasma	Reference
Crustaceans :—		
Carcinus maenas	4.0	Prosser (1950)
Carcinus maenas	6.0	,,
Cancer pagurus	3.49	,,
Cancer pagurus	4.37	,,
Eriphia	7.38	,,
Homarus vulgaris	2.25	,,
Homarus	4.0	,,
Pagurus	4.43	,,
Nephrops	4.2	,,
Molluscs :—		
Mya arenaria	0.09	Florkin (1949)
Helix pomatia	1.2–2.3	,,
Eledone moschata	10.0	,,
Pecten	0.075	Prosser (1950)
Solen ensis	0.12	,,
Mytilus edulis	0.145	,,
Aplysia	0.28	,,
Anodonta	0.074	,,
Pinna nobilis	0.028	,,
Helix	2.4 (summer), 3.3 (winter)	,,
Sepia	4.978	,,

Discussion

Crustacea :—It would be logical to expect that greater amounts of plasma proteins would increase the viscosity of the blood. The more viscous the blood, the more powerful the propelling mechanism should be. A comparison of the total protein values of the blood in the three crustaceans examined with those available in literature shows that there is a range of variation in this respect. The lowest value given by Prosser (1950) is for *Homarus vulgaris* (2·25). The value obatined by me for Paratelphusa is also low (2·206-2·83). Higher values are obtained for Panulirus and Scylla and those compare favourably with those given by Prosser for forms like *Cancer pagurus* (3·49-4·37), *Carcinus maenas* (4·0-6·0) and Eriphia (7·38). It may be of significance that the lowest figure available for crustaceans in this respect is that of Paratelphusa, a fresh-water form. It seems to be correlated with its fresh-water environment.

As regards the albumin globulin ratio, both Scylla and Panulirus which are marine decapods show a preponderance of albumin over globulin, whereas Paratelphusa which is a fresh-water form shows the reverse. Only a study of a number of forms will throw light on the significance of the partiality for globulin in some and albumin in others.

Mollusca :—The mollusca examined comprise lamellibranchs, gastropods and cephalopods. What is striking is that in the first group the amount of plasma proteins is very little, while it is more in the gastropods and still more in the cephalopods. Data available in the previous works also point to the same conclusion (Prosser 1950). The cephalopods being animals with a closed circulatory system and a powerful heart, it is no wonder that they have a large amount of plasma proteins in their blood. The heart beat in these animals is also reported to be high (Rogers 1938) and that also points to a blood with higher concentration of proteins in the plasma.

Amongst the lamellibranchs, the marine forms show more plasma proteins than the fresh-water form. This is true of gastropods also. A comparison between shelled forms and shell-less forms seems possible. The shell-less Aplysia has a greater amount of plasma proteins than the shelled-forms examined.

As regards the albumin/globulin ratio it is found that in all these molluscs the blood contains much more of globulin than albumin.

Comparison of crustacea and mollusca :—Taking into consideration the data presented here and available from previous works on the amount of plasma proteins in the blood in the two groups—crustaceans and the molluscs—it can be concluded that the crustaceans and the cephalopods with a well-established circulatory system comprising a heart, several blood vessels leading to extensive sinuses in the former and a capillary system in the latter and probably also with higher heart beats, have more concentration of plasma proteins in the blood than the lamellibranchs and gastropods. The latter groups seem to have evolved in the direction of fewer blood vessels and slow movement of blood and as a result probably of low metabolism.

Summary

(1) From the figures obtained from this study as well as those of others, it appears that the amount of plasma proteins in the crustacean blood varies considerably.

(2) The albumin/globulin ratio in the blood of crustaceans also seems to vary inasmuch as more of the former is present in Panulirus and Scylla and the reverse in Paratelphusa.

(3) The amount of plasma proteins in the blood in the lamellibranchs is little, more in gastropods and still more in cephalopods.

(4) In all the molluscs examined there is much more of globulin than albumin.

(5) The cephalopods and crustaceans with a well-laid-out circulatory system comprising a heart, several blood vessels leading to an extensive capillary system in the former and sinuses in the latter have more concentration of plasma proteins in the blood than the lamellibranchs and the gastropods.

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THE OXYPHORIC CAPACITY OF THE BLOOD OF SOME REPTILES AND MAMMALS

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CIRCULATION has been mentioned sometimes as a respiratory adaptation, and as such, the first and foremost function of the blood, in the majority of animals is the transportation and distribution of oxygen to the various tissues of the body. Allied to this, is yet another function equally important, the removal of carbon dioxide accumulated in the tissues and the transportation of the same to the organs of respiration where it is eliminated. It is well known that in certain very small invertebrate animals the oxygen carried in simple physical solution in the body fluid is sufficient to meet the metabolic needs of the animal. But as evolution progressed in the direction of complexity of organisation, the metabolic rate of animals increased. This led to the evolution of respiratory pigments capable of fixing, transporting and distributing greater quantities of oxygen to the tissues. However, in a few invertebrates (*e.g.*, *Urechis caupo*—Florkin 1949) the respiratory pigments are considered to be the storehouses of oxygen to be utilised only at times of need, the oxygen carried in physical solution in the blood being sufficient at normal times. But in the higher animals, particularly the vertebrates, the respiratory pigment is of vital importance for supplying oxygen for the immediate needs of the animal.

Much work has been done on the oxyphoric capacity of the blood of vertebrates and invertebrates. Hall and Gray (1929) determined the oxygen capacity of the blood of certain fishes. Erlanger (1921) determined the blood volume of mammals. Menon (1954) determined the iron in the blood of a few representative species of vertebrates. Prosser *et al.*, (1950) give data on the hæmoglobin content of a number of vertebrates. In this connection the work of Irving (1939) on the physiology of respiration of diving mammals is also important. A comparative study of the oxyphoric capacity of the blood of as many reptiles and mammals as possible will throw some light on the extent of the stepping up of the circulating fluid in that respect in the evolution of mammals from reptiles.

Material and Methods

In the present investigation the amount of iron in the blood of the following reptiles and mammals was ascertained, since the oxyphoric capacity of the blood is directly proportional to its iron content.

Reptiles	Mammals
(1) *Varanus griseus*	(1) *Suncus cæruleus*
(2) *Ptyas mucosus*	(2) *Felis domesticus*
(3) *Naja naja*	(3) *Pteropus giganteus*
(4) *Vipera russelli*	(4) *Macaca radiata*
(5) *Distira cyanocincta*	
(6) *Natrix piscator*	
(7) *Lissemys punctata*	
(8) *Geomyda trijuga*	

As hæmoglobin is distributed in the corpuscles, the efficiency of the blood in transporting oxygen to the tissues depends on the corpuscle volume and the nature and concentration of hæmoglobin in the corpuscles. Hence the percentage of corpuscles in the blood of the animals investigated has been determined. Also the amount of iron in the corpuscles has been calculated from the above values.

The corpuscle volume was determined by centrifuging a known volume of oxalated blood at a very high speed in a graduated centrifuge tube. The volume of corpuscles that settled down was noted and the percentage of corpuscles in 100 ml. of blood calculated from this.

The method of Kennedy as given by Hawk *et al.*, (1949) has been adopted for the determination of iron. The ferrous iron of the blood was converted to ferric iron and the organic matter present in the blood removed by digesting it with concentrated sulphuric acid and perchloric acid. The ferric iron was then converted to ferric thiocyanate by potassium thiocyanate and this was extracted by amyl alcohol. The colour thus produced was compared with that of a standard containing a known quantity of iron in a Klett-Summerson photo-electric colorimeter.

Results

Table I gives the corpuscle volume of the blood in the animals investigated. Table II gives the corpuscle volume in some animals, collected from previous works and presented for comparison. Table III gives the amount of iron in the whole blood of the animals studied, Table IV gives the amount of iron in 100 ml. of corpuscles, calculated from the average values of corpuscle volume and iron content of the blood.

Table I

The corpuscle volume of the blood

Name of the animal	Amount of corpuscles, by volume in 100 ml. of blood
Reptiles :	
1. Varanus	... ... 35.3 ± 4.8
2. Lissemys	... ... not estimated
3. Geomyda	... ... 26.7 ± 2.5
4. Ptyas	... ... 51.7 ± 12.0
5. Vipera	... ... 22.2 ± 3.6
6. Naja	... ... 23.8 ± 1.4
7. Distira	... ... 32.2 ± 1.1
8. Natrix	... ... 24.3 ± 5.8
Mammals :	
1. Suncus	... ... 47.5 ± 2.6
2. Pteropus	... ... 36.6 ± 4.4
3. Felis	... ... 30.7 ± 3.5
4. Macaca	... ... 39.4 ± 2.7

Table II

The corpuscle volume of the blood of some animals—data collected from previous literature

Name of the animal	Amount of corpuscle, by volume of 100 ml. of blood	Reference
Reptiles :		
Snapping turtle	... ... 20.4	Redfield (1933)
Alligator	... ... 14.5	,, ,,
Tortoise	... ... 9.8 to 22	,, ,,
Crocodile	... ... 18 to 24	,, ,,
Uromastix	... ... 28 to 29	Menon (1954)
Mammals :		
Rattus	... ... 36.6 to 41	,, ,,
Man	 46	Redfield (1933)
Horse	 36	,, ,,
Sheep	 39	,, ,,
Rat	 47	,, ,,
Sea lion	 29	,, ,,
Porpoise	 35	,, ,,

Table III

The amount of iron in the whole blood

Name of the animal	Amount of iron in mg. per 100 ml. of blood
Reptiles :	
1. Varanus	31.582 ± 5.792
2. Lissemys	22.977 ± 3.626
3. Geomyda	24.530 ± 4.456
4. Ptyas	35.279 ± 3.136
5. Vipera	22.708 ± 2.17
6. Naja	21.182 ± 1.746
7. Distira	33.247 ± 3.864
8. Natrix	22.165 ± 3.933
Mammals :	
1. Suncus	49.894 ± 6.601
2. Pteropus	43.654 ± 4.02
3. Felis	36.10 ± 4.961
4. Macaca	31.709 ± 2.340

Table IV

The amount of iron in 100 *ml. of corpuscles*

Name of the animal	mg. of iron per 100 ml. of corpuscles
Reptiles :	
1. Varanus	90.675
2. Lissemys	not calculated
3. Geomyda	85.392
4. Ptyas	66.610
5. Vipera	96.090
6. Naja	88.128
7. Distira	104.446
8. Natrix	96.033
Mammals :	
1. Suncus	102.520
2. Pteropus	117.909
3. Felis	118.590
4. Macaca	80.296

Discussion

An examination of the tables shows that the blood of mammals in general has a greater volume of corpuscles in the blood (Table I), a greater percentage of iron in the whole blood (Table III) and a greater concentration of iron in the corpuscles (Table IV) than that of reptiles. This general statement, however, is not found to be applicable to Felis with regard to corpuscle volume and to Macaca with regard to the other two characteristics. I should mention, however, in this connection

that the values given by Rao and Rao (1940) for *Macacus sinicus* (14·1 g. of hæmoglobin/100 ml. of blood and 32·54 g. of hæmoglobin/100 ml. of corpuscles) and Shukers *et al.*, (1938) for *M. mulatta* (12·2 g. of hæmoglobin/100 ml. of blood and 30·5 g. of hæmoglobin /100 ml. of corpuscles) indicate a higher percentage of iron in the whole blood and an equally high amount of concentration of iron in the corpuscles. These higher values obtained for mammals compared to reptiles are in agreement with the findings of Florkin (1949), Redfield (1933) and Menon (1954).

Compared to the available values for the corpuscle volume (Tables I and II) and iron content (Table III) in the blood of other mammals, those obtained for Suncus seem to be high. The metabolic rate of small animals and in particular that of shrews (Prosser 1950) is very high. Therefore this high corpuscle volume and iron content can perhaps be attributed to their higher metabolic rate.

The amount of iron obtained for Varanus (Table III) compares favourably with that obtained by Menon (1954) for Uromastix. The corpuscle volume and the iron content of the blood of Felis, however, is lower than that reported for other mammals. The concentration of hæmoglobin in the corpuscles, however, as judged from its iron content is found to be high in Felis (Table IV). This seems to be a compensation for the low corpuscle volume as well as iron content of the blood of Felis.

The snakes Natrix, Naja and Vipera are characterised by a low volume of corpuscles, a low iron content and an average concentration of iron in the corpuscles. Compared to them Ptyas shows a very high corpuscle volume (Table I) in its blood, the highest value obtained among the animals investigated. But in the amount of iron in 100 ml. of corpuscles it shows the least value (Table IV). A larger corpuscle volume imparts greater oxyphoric capacity to the blood (Florkin 1949) as the surface area available for diffusion of gases is greater. A larger amount of iron enables the animals to fix more oxygen. The low concentration of iron in the corpuscles in Ptyas seems to have been compensated by its larger corpuscle volume.

Compared to Natrix, Naja and Vipera, Distira is characterised by a larger volume of corpuscles, a greater percentage of iron and a fairly high concentration of iron in the corpuscles. Irving (1939) has reported an increased concentration of corpuscles and a large oxygen capacity to the blood of diving mammals compared to land ones. Distira is capable of remaining submerged under water continuously for several hours. Ditmars (1946) mentions that it is the case with all sea snakes. Hence the higher quantity of corpuscles in Distira should be correlated with its capacity to remain under water for a long time.

Natrix though frequents water and is capable of remaining under water for a short while, under ordinary conditions it does not remain submerged in water. The low corpuscle volume of Natrix which is similar to that of Naja and Vipera is therefore not surprising. Varanus occupies a superior position in corpuscle volume but is similar in other respects to the land snakes.

The corpuscle volume of Lissemys could not be ascertained on account of the fact that the blood lakes as soon as it is mixed with oxalate.

The iron content and the concentration of iron in Macaca is lower than expected. More determinations have to be made to see if the present values can be accepted.

In conclusion it may be stated, that even though the iron content and the corpuscle volume in the blood of Mammals show a general increase compared to those of reptiles, within each class the values show marked variations. some animals attaining greater specialisation than others.

Summary

(1) The larger corpuscle volume and greater iron content in the blood of Suncus can perhaps be attributed to the higher metabolic rate expected in small animals especially shrews.

(2) The low concentration of iron in the corpuscles is compensated in Ptyas by its larger corpuscle volume.

(3) The larger corpuscle volume and the greater iron content in the blood of Distira can be explained as an adaptation to remain submerged under water for prolonged periods.

(4) In the three respects evaluated (corpuscle volume. iron content in the whole blood and the concentration of iron in the corpuscles) the mammals excel the reptiles, but there are low and high levels in both groups.

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A COMPARATIVE STUDY OF THE BLOODS OF SOME CRUSTACEANS, MOLLUSCS AND VERTEBRATES WITH REFERENCE TO THEIR CONSTITUENT AMINO-ACIDS

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DIFFERENT proteins call for different combinations of amino-acids. Hence the assortment of amino-acids obtained after hydrolysis of a protein will depend on the character of the protein used. It is also known that the quantitative yields of the various amino-acids, from different proteins show a wide range of variation. Roche comparing the amino-acid composition of hæmoglobins and erythrocrurins states that the latter contain very much larger amounts of arginine and cystine but less histidine and lysine (as cited in "Biochemical evolution" by Florkin 1949).

In the present paper the amino-acids obtained in the blood hydrolysates in a few crustaceans, molluscs and vertebrates are given.

Material and Methods

The following animals were chosen from the three groups :—

Crustacea :

Panulirus polyphagus
Paratelphusa guerini
Scylla serrata

Mollusca :

Telescopium telescopium
Lamellidens lamellidens
Pila globosa

Vertebrata :

Lissemys punctata
Columba livia
Rattus rattus

Hydrolysis :—About 10 c.c. of oxalated blood was refluxed on a sand bath for 36 hours, with 50 cc. of 6N hydrochloric acid (analar). The hydrolysed material was made free from excess of hydrochloric acid by heating on a steam bath. Its pH was then adjusted very carefully to 6 with the help of paper indicator. The hydrolysate was filtered and its volume was made up to 50 cc. with distilled water.

Circular paper chromatography :—Separation and identification of amino-acids were done by Rutter's technique with Giri and Rao's modification (1952). The details of procedure were the same as described by Rangnekar and Muthe (1954).

Ascending strip chromatography :—The presence of the various amino-acids was confirmed with the help of ascending strip chromatogarphy. The experimental procedure described by Airan *et al.*, (1953) was followed.

Results

The following table gives the results obtained.

Table 1

The total amino-acids obtained from the blood hydrolysates

Amino acid	Para-telphusa	Panu-lirus	Scylla	Teles-copium	Lamelli-dens	Pila	Lisse-mys	Col-umba	Rattus
Leucine	+	+	+	+	+	+	+	+	+
Phenylalanine ...	+	+	+	+	—	+	+	+	+
Valine-methionine	+	+	+	+	+	+	+	+	+
Tyrosine	+	+	+	+	—	+	+	+	+
Proline	+	+	+	+	+	+	+	+	+
Alanine	+	+	+	+	+	+	+	+	+
Glutamic acid-threonine ...	+	+	+	+	+	+	+	+	+
Glycine serine-aspartic acid ...	+	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+	+	+
Histidine	+	+	+	+	+	+	+	+	+
Lysine	+	+	+	+	+	+	+	+	+
Cystine	+	+	+	+	+	+	+	+	+

Discussion

The HCl hydrolysate obtained from the bloods of crustaceans as well as molluscs except Lamellidens and from the vertebrates yielded in chromatography identical assortment of the following amino-acids :—

Leucine, methionine, phenylalanine, valine, tyrosine, proline, alanine, glutamic acid, threonine, glycine, serine, aspartic acid, arginine, histidine, lysine and cystine. In Lamellidens bands corresponding to phenylalanine and tyrosine were not represented. In an earlier paper (1952) I had pointed out that the blood of Lamellidens is colourless and suggested that a colourless protein containing iron might be present. The present chromatographic study also emphasises the difference in composition between Lamellidens blood and those of other molluscs.

The blood of Rattus, however, indicated the presence of an additional amino-acid the identity of which could not be settled. Its position was just below the band corresponding to tyrosine in the ascending strip chromatogram.

Summary

1. The HCl hydrolysate obtained from the blue as well as red bloods yielded in both ascending strip as well as circular chromatograms the same assortment of amino-acids.

2. The colourless blood of Lamellidens did not indicate the presence of phenylalanine and tyrosine. This is another proof that the blood of Lamellidens differs from that of other molluscs in its composition.

Acknowledgment

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A COMPARISON OF THE GROSS CHEMICAL COMPOSITION OF THE RED AND WHITE MUSCLES IN THE TWO FISHES, SCATOPHAGUS ARGUS AND LABEO ROHITA

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THE skeletal muscles of the vertebrates can be roughly classified according to colour into red and white. Both types occur usually in different parts of the same animal. The red muscle usually occurs in regions requiring long and sustained contractions as the flight muscles of birds, the diaphragm and the gluteal muscles of running mammals etc. In certain fishes also, there exists a band of red muscle along the active lateral-line region and slightly diffused at the pectoral and caudal fin regions.

Some literature is available concerning the chemistry of the red and white muscles. Needham (1926) in a review of the former has treated all the previous work exhaustively. Recently George (1952) has dealt with this aspect of the vertebrate muscles and has given some interesting data. But an account of the gross chemical composition of these muscles in fishes, however, is lacking. The present paper deals with a comparative study of the gross chemical composition of the red and white muscles in the two fishes.

Material and Methods

The fishes selected for this study were *Scatophagus argus* and *Labeo rohita*. In both the fishes the red muscle could be easily discerned as a band along the lateral-line region and slightly scattered along the pectoral and caudal fin regions.

The total muscle proteins were estimated by a slightly modified form of Wong's (1923) method. Fat was extracted by ether using a Soxhlet apparatus and weighed after evaporating the ether. Iron was determined by Kennedy's (1927) method. The amount of carbohydrates was calculated by subtracting the amounts of fat, proteins and ash from the total solids. Qualitative and quantitative estimations of total amino acids were carried out by the circular paper chromatographic method.

The determinations were in each case carried out five times using every time a different specimen and finally the mean of the values calculated therefrom.

Results

The following tables give the mean values obtained for the five determinations carried out :—

Table I

The gross chemical composition of the red and white muscles.

Name of the animal	Colour of the muscle	Water	Total solids	Proteins	Fat	Ash	Iron (mg. per 100 g. of wet muscle)	Carbohydrates
Scatophagus argus	Red	56.529	43.471	17.635	24.522	1.004	6.60	0.310
	White	77.436	22.564	16.752	4.001	1.374	0.83	0.437
Labeo rohita	Red	72.479	27.521	18.140	8.063	1.016	8.48	0.302
	White	81.080	18.920	16.771	0.303	1.412	1.50	0.434

Table II

The amount of amino acid expressed in μg. of nitrogen in 15 μ. of nitrogen of the hydrolysate.

Name of the amino-acid	*Scatophagus argus*		*Labeo rohita*	
	Red	White	Red	White
1. Leucine	1.26	0.85	1.33	0.97
2. Phenyl alanine	0.26	0.21	0.28	0.21
3. Tyrosine	0.13	0.13	0.36	0.31
4. Alanine	0.99	0.77	1.19	0.91
5. Arginine	1.30	0.63	1.09	0.55
6. Histidine	1.14	0.85	1.28	0.87
7. Lysine	2.00	1.25	1.56	0.94
8. Cystine	0.12	0.07	0.12	0.09

Discussion

The water content of the red muscle of the two fishes investigated was markedly low and the total solids very high compared to those of the white muscle of the same fishes. The red muscle of Scatophagus has 56·529% of water and 43·471% of total solids in comparison to 77·436% and 22·564% of the white muscle. But the corresponding values of water in Labeo are higher. These figures suggest that there is a marked reduction in the amount of water present in the red muscle and correspondingly the amount of total solids is more.

As regards the total muscle proteins, in both the fishes the red muscles have only a slightly greater amount of proteins than the white which may not be significant. Previous investigators like George (1952) and Fukui (1953) have also obtained similar results.

A casual glance over table (1) shows that the difference between the fat content of the red and white muscles in these two fishes is sufficiently great to merit recognition. The red muscle of Scatophagus shows 24·522% in comparison with 4·001% in its white muscle. The red muscle of Labeo has lower value, the red muscle having 8·063% compared to 0·303% in the white muscle. Greene (1926) found 30% fat in the red muscle of King Salmon, before its spawning migration. These figures suggest that fat is often deposited in large amounts in the red muscles of fishes.

There exists a striking contrast also in the iron content of the red and white muscles. Red muscle of Scatophagus has an iron content of 6·60 mg. compared to 0·83 mg. in the white muscle. In Labeo the red muscle has a greater amount of iron (8·48 mg.), while the white muscle has only 1·50 mg. The red muscle of fishes by virtue of its comparatively higher iron content presents a striking parallel to the avian muscle. Since the amount of iron present in a muscle is a rough indication of its myoglobin content, the greater amounts of iron present in the bands of red muscle in fishes may be regarded as a reflection of the presence of that substance in large quantities.

What purpose this extra fat and iron in the red muscle serve, is as it stands a matter of conjecture. Since it is distributed along the lateral-line and the bases of fins, it can be surmised that it is perhaps utilized for locomotion.

The Carbohydrates and Ash content do not depict any significant variation. The amino-acids obtained by the hydrolysis of the red and white muscles of the fishes are more or less similar.

Summary

(1) The gross chemical composition of the Red and White muscle in two fishes *Scatophagus argus* and *Labeo rohita* was studied.

(2) There is a marked reduction in the water content of the red muscle in comparison with the white and a corresponding increase in the total solids is also observed.

(3) Fat is present in very large amounts in the red muscle of the fishes as was noted in that of birds. The location of the red muscle along the lateral-line tract and also at the base of the fins, suggests that its fat may be utilised in locomotion.

(4) The red muscle also contains more of iron than the white which indicates the presence of myoglobin. This finding also supports the suggested utilization of fat in locomotion.

(5) The values obtained for ash, carbohydrates and total amino acids do not seem to exhibit any significant variation.

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I wish to express my grateful thanks to Dr. C. J. George, Head of the Department of Biology, Wilson College, Bombay, for his constant guidance and encouragement during the progress of this work. My thanks are also due to the authorities of the Taraporevala Aquarium for the supply of fishes for this investigation.

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THE ALBUMIN TO YOLK RATIO IN MARKET EGGS FROM BOMBAY

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THE amounts of dry albumen and yolk from 40 hen's eggs taken at random from the Bombay market were determined to find out if there existed any variation in the ratio of dry albumen to yolk.

Method

Each egg was broken open in a small bowl and the albumen was separated from yolk and both kept in previously weighed dry petri dishes in an oven at 80°C. for dehydration. Care was taken not to lose any part of albumen and that sticking on to the bowl was rinsed out with distilled water and poured into the albumen dish. After about 12 hours of heating the dishes with their contents were transferred to a desiccator for cooling and weighed. This process was repeated till constant weights were obtained. Since the weights of the dishes were known, the weights of dried albumen and yolk could be arrived at.

Results

The weights of dried albumen and yolk in the 40 eggs as well as the relative percentage of albumen to yolk are given in the table below.

Table I

	Wt. of dry Albumen	Wt. of dry Yolk	Relative percentage of Albumen to Yolk		Wt. of dry Albumen	Wt. of dry Yolk	Relative percentage of Albumen to Yolk
1	2.270 gms.	4.781 gms.	47.4	21	2.969 gms.	7.993 gms.	40.15
2	1.826 gms.	4.478 gms.	40.7	22	2.315 gms.	7.662 gms.	32.5
3	3.078 gms.	4.855 gms.	63.3	23	2.546 gms.	6.216 gms.	40.9
4	1.483 gms.	4.848 gms.	30.5	24	2.859 gms.	7.016 gms.	40.7
5	2.451 gms.	4.978 gms.	49.2	25	2.705 gms.	6.884 gms.	39.2
6	2.56 gms.	5.888 gms.	43.5	26	2.067 gms.	7.522 gms.	27.4
7	1.793 gms.	6.075 gms.	29.5	27	3.316 gms.	8.806 gms.	37.6
8	2.295 gms.	4.398 gms.	52.1	28	2.795 gms.	7.130 gms.	39.2
9	1.754 gms.	5.012 gms.	34.9	29	2.471 gms.	7.9 gms.	31.2
10	1.80 gms.	5.069 gms.	35.5	30	3.142 gms.	7.6 gms.	41.3
11	1.825 gms.	4.145 gms.	44.0	31	3.575 gms.	7.825 gms.	45.6
12	1.9 gms.	3.543 gms.	50.8	32	2.913 gms.	7.044 gms.	41.3
13	1.822 gms.	3.983 gms.	45.7	33	2.745 gms.	7.523 gms.	36.5
14	2.168 gms.	5.193 gms.	41.7	34	2.862 gms.	8.11 gms.	35.2
15	2.034 gms.	4.17 gms.	45.0	35	2.941 gms.	6.52 gms.	45.1
16	2.202 gms.	3.767 gms.	58.4	36	2.852 gms.	7.245 gms.	39.2
17	2.392 gms.	4.335 gms.	55.1	37	2.591 gms.	6.587 gms.	39.3
18	1.925 gms.	4.854 gms.	39.6	38	2.624 gms.	7.835 gms.	33.4
19	1.957 gms.	4.575 gms.	42.3	39	2.915 gms.	5.732 gms.	50.8
20	1.75 gms.	4.296 gms.	40.7	40	3.097 gms.	7.969 gms.	38.9

Discussion

The results of the examination of 40 eggs given in the table above show that the variation of albumen to yolk ranges from 27·4 to 63·3. Since such a wide variation is found in the relative amounts of albumen and yolk, it follows that the nutritive values of these eggs also differ considerably. It is suggested that such a wide difference is perhaps traceable to the fact that the local poultry is not composed of a homogeneous stock.

Acknowledgment

I wish to express my grateful thanks to Dr. C. J. George, Head of the Department of Biology, Wilson College, for suggesting the line of investigation and for his constant guidance throughout the work.

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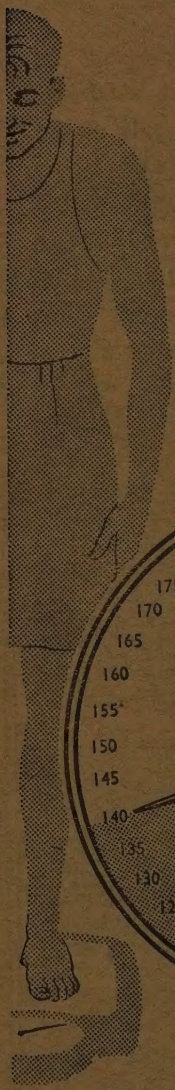

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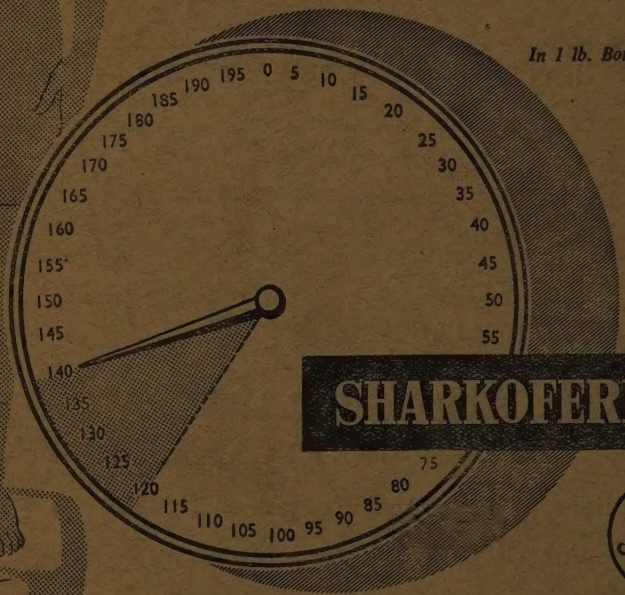

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